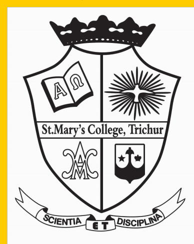
December 2017 Volume 8 Number 1

Annals of Basic and Applied Sciences

ISSN 2277 – 8756

ABAS
December
2017
Vol. 8, No. 1



A Peer Reviewed Journal

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Annals of Basic and Applied Sciences

December 2017, Vol. 8, No. 1, ISSN 2277 – 8756.
Publication of St. Mary's College, Thrissur-680020, Kerala, India.

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Qualitative evaluvatuion on phytochemical, antimicrobial and antioxidant profiles of Ethanolic extract of *Carica papaya*

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Abstract

Ayurveda is a traditional Indian medicinal system being practiced thousands of years. Considerable research on pharmacology, chemistry and clinical therapeutics has been carried out on ayurvedic medicinal plants. The nontoxic or less toxic nature and the lesser known side effects of plant products has increased the popularity of plant products as treatment modalities for various ailments. Traditionally, leaves of ***Carica papaya*** have been used for treatment of a wide range of ailments, like in treatment of malaria, dengue, jaundice. The present study was designed to observe antimicrobial, antioxidant activities of the ethanolic extract of ***Carica papaya***. Phytochemical analysis of extract was carried out.

Keywords: *Ayurvedic medicines, Medicinal plants, Antimicrobial study*

1.Introduction

Around the world, at least thirty five thousand plant species are used for medicinal purposes and virtually all plant parts are usually consumed as food for efficient supply of energy (Kong et al., 2003). The study of disease and their treatment have been existing since the beginning of human civilization. Plant kingdom is one of the major search areas for effective works of recent days. The importance of plants in search of new drugs is increasing with the advancements of medical sciences.

Despite the advent of modern medicine, the popularity of plant natural products as treatment modalities for various ailments has increased worldwide due to their nontoxic or less toxic nature and the lesser known side effects than the modern generic drugs. The burden infectious diseases is a big challenge and nuisance to human health and

responsible for certain deaths on daily basis. Plants have now scientifically proven as effective, cheaper alternative sources and have very least side effects than commercially available synthetic drugs. *Carica papaya Linn* is commonly called as paw-paw and it belongs to the family *Caricaceae*. Papaya possesses excellent medicinal properties for treatment of different ailments. (K. Kayalvizhi et al., 2015)

The plant is native to tropical America and was introduced to India in 16th century. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves. Both leaf and fruit of the Carica papaya Linn. possess carotenoids namely β- carotene, lycopene, anthraquinones, glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepato protective, wound healing, recently its antihypertensive and antitumor activities have also been established. Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc. The different parts of the *Carica papaya* plant including leaves, seeds, latex and fruit exhibited to have medicinal value.

2. Methodology

2.1 Collection of leaves

Fresh leaves of *Carica papaya* were collectd from outskirts of Thrissur, Kerala, India. The plant was identified at Dept. of Botany, St Mary's College ,Thrissur Kerala, India. The fresh leaves were harvested properly washed in tap water, and then rinsed in sterile distilled water. The leaves were dried in the hot air oven at 40° C for six hours. The dried leaves were then crushed into powder using electric grinder to obtain a powered form. The powdered samples were stored in airtight glass containers , prior to analysis.

2.2 *Preparation of ethanolic extract*

The crude powdered sample of 150 g was defatted with petroleum ether in a soxhlet apparatus for 6-8 hours, repeatedly thrice. The ethanol extraction was done sequentially. The dried samples were then stored in air tight bottles at 4^{0}C.(Baku, 2007)

2.3 Phytochemical Screening

The ethanolic extract was analysed for the presence of various phytochemicals by the standard procedure of Sofowara (1993);Trease and Evans(1989);and harborne(1973)

2.4 Antibacterial activity

Antibacterial test were carried out by the disc diffusion method with some modification .The bacterial cultures used for the test *were Pseudomonas sp, Bacillus sp, Klebsiella sp, Escherichia coli, Salmonella sp, Serratia sp and Proteus sp.* From the overnight culture, 0.1 ml of culture was uniformly distributed onto MHA plates. A filter paper disc of 6 mm diameter was punched out from a Whatmann No: 1 filter paper and sterilized. Then the discs were placed on the surface of Mueller Hinton agar plates at a distance of 2 cm using sterile forceps. Drugs of different concentrations (50,100,150,200,250 mg/ml) and a control (2 % DMSO) were added on each disc with a micropipette. Then the plates were incubated at 37˚c for 24-48 hrs. After incubation zone diameter was measured.

2.5 Antioxidant activity

2.5.1Total anti-oxidant capacity

The total anti-oxidant capacity was measured according to spectrophotometric method of Preito (Preito et al.,1999) . 0.1 ml of the extract (10 mg/ml)was dissolved in water was combined in an eppendorf tube with 1 ml of reagent solution(0.6 M H_2SO_4, 2.8mM Sodium phosphate and 4mM Ammonium molybdate).The tubes were capped and incubated in a thermal block at 95˚C for 90 minutes. After cooling to room temperature, the absorbance of the aqueous solution was measured at 695 nm against blank .Vitamin C used as standard.

2.5.2 Hydroxyl radical scavenging activity

Hydroxyl radicals generated from Fe 3+ ascorbate-EDTA- H_2O_2system(Fenton's reaction) was eliminated by its degradation of deoxyribose that resulted in thiobarbituric acid reaction substance(TBARS) (Elizabeth and Rao,1990).The reaction mixture contained deoxyribose (28 mM);

Fe Cl3(1mM);KH2Po4 –KOH buffer(20 Mm ,pH 7.4); EDTA (1 mm); H2O2(1 Mm);Ascorbic acid (0.1 Mm) and various concentrations of drug in a final volume of 0.1 ml. The reaction mixture incubated for 1 hour at 37˚C.The TBARS formed was estimated by TBA method. (Ohkawa et al.,1979).The hydroxyl radical scavenging activity was determined by comparing absorbance of control with that of treatments. Vitamin C was used as standard.

2.5.3 DPPH free radical scavenging assay

In this method a commercially available and stable free radical 2, 2, diphenyl-1-picryl hydrazyl ($DPPH^+$), soluble in methanol was used(Aquino et al.,2001). DPPH in its radical form has an absorption peak at 515 nm, which disappeared on reduction by an antioxidant compound .An aliquot (25 µl) of the extracts was added to 1 ml of freshly prepared DPPH solution(0.25g/l in methanol).The sample were kept for 20 minutes in dark and the decrease in absorbance was measured at 515 nm. Vitamin C was used as the standard.

3.Results

Table 1:- Phytochemical profiling of ethanolic extract of *Carica papaya*

Phytochemicals	Ethanolic
Alkaloids	+
Anthraquinones	_
Carbohydrates	+
Cardiac glycosides	_
Coumarin	+
Flavanoids	+
Phenols	_
Phlobatannins	_
Proteins	_
Quinone	_
Saponin	_
Tannins	_

Table 2:-Antibacterial Activity of *Carica papaya* by Disc Diffusion method

Name of the extract	Concentration of the extract (mg)	Diameter of Zone of inhibition (MEAN ± SD)					
		Pseudomonas sp	*Serratia sp*	*Bacillus sp*	*Salmonella sp*	*E.coli*	*Klebsiella sp*
Ethanolic extract	50 mg	NA	NA	NA	NA	NA	NA
	100 mg	7.5±0.70	NA	7.5±0.70	NA	NA	NA
	150 mg	12±1.41	NA	12±1.41	NA	NA	NA
	200 mg	11±1.41	9.5±0.70	11±1.41	7.5±2.12	10±1.41	9.5±0.70
	250 mg	11.5±0.70	13±1.41	11.5±0.70	13.5±0.70	13.5±9.19	13±1.41

Table 3 Antioxidant activity of Ethanolic extract of *Carica papaya* by Total Antioxidant Assay

Concentration of extract (µg/ml)	Percent activity (MEAN ± SD)
	Ethanolic extract
50	**4.49±1.21**
100	**14.48±1.95**
150	**19.69±0.86**
200	**40.60±0.70**
250	**53.19±0.43**

NA * No activity

Table 4 Antioxidant activity of Ethanolic extract of *Carica papaya* by DPPH Free radical scavenging activity

Concentration of extract (µg/ml)	Percent activity (MEAN ± SD)
	Ethanolic extract
50	20.1±0.25
100	24.09±0.84
150	26.7±0.07
200	28.07±0.84
250	29.42±0.17

NA * No activity

Table 5 Antioxidant activity of Ethanolic extract of *Carica papaya* by Hydroxyl Radical Scavenging Activity

Concentration of extract (µg/ml)	Percent activity (MEAN ± SD)
	Ethanolic extract
50	11.41±0.30
100	16.7±0.15
150	33.07±0.14
200	34.69±0.30
250	44.28±0.14

4. Discussion and Conclusion

Herbs have been a source of medical compounds since time immemorial. History of use of herbal medicine in treatment of disease can be identified with the history of medicine and with the history of civilization itself. All parts of plants were used in the Ayurvedic ,Unani Allopathic system of medicines for the treatment of a number of human diseases. The current study show that ethanolic extracts of *Carica papaya* posses significant antioxidant properties. Phytochemical analysis showed the presence of large number of biological active plant constituents. Phytochemicals such coumarin, saponin, cardiac glycosides, tannins, phenols, flavanoids, alkaloids, carbohydrates were detected in the ethanolic extract. Ethanolic extract of *Carica papaya* was found to be effective against *Pseudomonas sp, Bacillus sp and E coli.* The maximum inhibitory activity was against *E coli.*

The antioxidant activity for the extract of *Carica papaya* were conducted using Total Antioxidant Assay, DPPH ,Hydroxyl Radical Scavenging Assay. The antioxidant activity was observed in dose dependent manner. *Carica papaya* have active components which are useful in the straitening of antioxidant properties (Kaleem et al.,2005).This was moderately correlated to the high phenolic content in the herb. However the studies reveals that *Carica papaya* can be a potential and effective source for drug discovery. Further research may pave way to the development of effective antioxidant and antibacterial agents from *Carica papaya*.

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Antibacterial Effect of Citrus Fruit Juice against Enteric and Non Enteric Pathogenic Bacteria

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Abstract

The present study was carried out to determine the antimicrobial effect of three different citrus fruit juices against four enteric and two non enteric pathogens named Escherichia coli , Salmonella , Proteus, Klebsiella ,Vibrio,and Staphylococcus species. The study was done by well diffusion and disc diffusion method. The use of different concentrations (100%, 75%, 50%, 25%) ofcitrus juice extracts had an effective antibacterial activity. Lemon juice was the most effective against the test organisms in both undiluted and diluted concentration. Pomelo juice showed antimicrobial activity only against Vibrio and Salmonella.Orange juice showed antimicrobial activity only against Staphylococcus and Escherichia coli .

Key Words : citrus fruit , well diffusion , disc diffusion , antimicrobial effect

1. Introduction

Bacterial infections are among the important infectious diseases. Hence, over 50 years of extensive researches have been launched for achieving new antimicrobial medicines isolated from different sources. Despite progress in development of antibacterial agents, there are still special needs to find new antibacterial agents due to development of multidrug resistant bacteria(R.Wise,T.Hart,O.Cars et al. ,1998)

The emergence of resistant organisms presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity.The increase in antibiotic resistance bacteria is largely due to the widespread use of antibiotics

in medicine in animal care and agriculture (Bansode et al 2012).)Citrus fruits have been of interest for extraction of antimicrobial metabolites by large number of researchers (Kumar et al., 2011; Kumar et al., 2010; Amandeep et al., 2009) but the peels have been less studied. Lemon juice has been used in the treatment of oral thrush in HIV/AID patients (Wright et al., 2009). The antioxidant activities of citrus flavonoids and phenolic compounds exhibited a potent antibacterial activity which is probably due to their ability to complex with bacterial cell walls and disrupt microbial membrane . Studies have shown that concentrated or freshly squeezed lemon juice has antibacterial activity against Vibrio species (Tomotake et al., 2006), Antibacterial properties of plant extract have been a hot topic for the researchers. Besides plants, fruits also have been studied by the researchers for the presence of bioac-tive compounds close related to herbs, commonly referred as phytochemicals such as tannins, carotenoids, polyphe-nols and anthocyanins (Khushwaha et al 2012). The current research focuses on the extraction and assay of antibacterial component from citrus fruit which are easily available at very low cost.

2. Materials and Methods

2.1 Bacterial cultures - *Escherichia coli , Salmonella , Proteus, Klebsiella ,Vibrio,* and *Staphylococcus species.* were kindly provided from Poly Clinic Pvt Ltd, Thrissur , Kerala , India.

2.2.Citrus fruits – Fresh lemon ,Orange , Pomelo were obtained from local market of Thrissur.

2.3 Taking of juice from fruits

Surface of the fruit was disinfected using ethanol. Fruit was pierced and juice was aspirated and collected in beaker in sterile condition.

2.4 Preparation of different concentrations of fruit juice

Different concentrations were made by adding sterile physiological saline(0.85%) into the juice. To prepare 1ml of 25% concentration of fruit juice,0.25 ml juice was added to 0.75 ml of saline, 1ml of 50% concentraton,0.5 ml juice was added to 0.5ml saline, for 1ml of 75% concentration ,0.75 ml of juice was added to 0.25ml of saline,and for 1 ml of 100% concentration,1ml juice was used.The physiological saline was used as control.

2.5. Anti-bacterial activity testing by Well diffusion method

Antibacterial test were carried out by the well diffusion method. Overnight bacterial cultures were diluted in the nutrient broth to obtain a bacterial suspension of 10^8 CFU/ml. Petriplates containing 20 ml of Muller-Hinton Agar media were inoculated with diluted cultures with a sterile cotton swab is dipped into standardized bacterial test suspension and used to evenly inoculate the entire surface of the Muller-Hinton Agar plates. After the agar surface has dried for about 5 minutes, five wells, all are have equal diameter were made in the plate at equal distance. Citrus fruit juice of different concentrations (25,50,75,100µl)and a control (0.85%) saline were poured on each well by using micropipette. Plates were incubated at 37^0C for 24h .The antibacterial activity was determined by measuring the inhibition zone.

2.6. Anti-bacterial activity testing by Agar disc diffusion assay

The antibacterial activity of the citrus fruits extracts was determined by the disc diffusion method.Briefly ,overnight bacterial culture were diluted in the Muller-Hinton broth to obtain a bacterial suspension of 10^8 CFU/ml. Petriplates containing 20ml of Muller-Hinton Agar media.A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Muller-Hinton Agar plate.After the agar surface has dried for about 5 minutes , Citrus fruit juice of different concentrations (25,50,75,100µl) were loaded on to the filter paper discs (whatman No.1 ,6mm diameter) were placed on the inoculated agar surface were allowed to dry completely. Standard saline (0.85%) was placed as control. Plates were incubated at for 37^0C 24h. The antibacterial activity was determined by measuring the inhibition zone. (S.Sundar and koilpillai, 2015).

3. Result and Discussion

From three citrus fruits used in the study for evaluation of their antimicrobial activity against four enteric bacteria and two non enteric bacteria initially determined by Muller Hinton Agar well diffusion method. All of the isolated species showed activity against citrus fruit juices. Juice of *Citrus lemon*

showed highest inhibitory effect against *Vibrio* species with largest DIZ (Diameter of Inhibition Zone) value followed by *Staphylococcus* species ,*Salmonella* species and *Proteus* species. Juice of *Citrus maxima* showed highest inhibitory effect against *Vibrio* species followed by *Salmonella* species. *de Castillo etal* (2000) also reported that freshly squeezed lemon juice inhibited the growth of *V. Cholerae* . Juices of *Citrus sinesis* showed highest inhibitory effect against *Staphylococcus* species followed by *E.coli.*

The antibacterial activity of these pathogens are further determined by disc diffusion method. All isolated bacteria showed susceptibility against citrus fruit juices. Juice of *Citrus limon* showed highest inhibitory effect against *Vibrio* species with largest DIZ followed by *Staphylococcus* species ,*salmonella* species and *Proteus* species. Juice of *Citrus maxima* showed highest inhibitory effect against *Vibrio* species followed by *Salmonella* species. *Citrus sinesis* showed highest inhibitory effect against *Staphylococcus* species followed by *E.coli* . *Citrus limon* showed highest inhibitory activity against *Vibrio* species while lowest activity against *Proteus* species. The results showed in Table 1 and Table 2.

Similar results for the various extracts from citrus fruits have been reported by many authors (Kumar et al., 2011; Kumar et al., 2010; Amandeep et al., 2009; Nurmahani et al., 2012) .On comparison of antimicrobial activity of different citrus fruits the results showed that *Citrus limon>Citrus maxima>citrus sinensis*. The results of Table 1 and Table 2 revealed that *Citrus limon* juice showed highest activity against almost all enteric pathogens and non enteric pathogens. From the results obtained, the highest inhibitory effect showed by 100µl concentration and least effect by 25µl.

4. Conclusion

The study summarised here demonstrate that the microbial load of human body constitute enteric and non-enteric pathogens.The bacterial isolates identified in this study are common human enteric and non-enteric pathogens.This study emphasizes the

need of usage of citrus fruits in our diet.Because the results showed that the citrus fruit juice have high antibacterial activity against almost all enteric and non-enteric pathogens .The well diffusion and disc diffusion methods using citrus fruit juice showed antibacterial activity against *Vibrio, Salmonella, Staphylococcus, E.coli, Proteus* and *Klebsiella*..The study points out that the infections with enteric and non-enteric pathogens can be successfully eliminated by the use of easily available citrus fruit juices. This approach however go a long way in combining the rising tide of antibacterial resistance.

Table 1: Showing Antibacterial Activity By Well Diffusion Method

Bacteria	Diameter Of Inhibition Zone in mm.												
	Citrus limon				*Citrus maxima*				*Citrus sinensis*				
	100μl	75μl	50μl	25μl	100μl	75μl	50μl	25μl	100μl	75μl	50μl	25μl	
Enterobacteriacea													
E.coli	15	10	11	7	------	-----	-----	-----	7	4	2	-----	------
Salmonella	21	19	17	14	18	14	10	-----	------	-----	-----	-----	------
Proteus	19	17	15	11	------	-----	-----	-----	------	-----	-----	-----	------
Klebsiella	13	11	10	3	------	-----	-----	-----	------	-----	-----	-----	------
Non-Enterobacteriacea													
Vibrio	23	22	18	16	21	18	13	-----	-----	-----	-----	----	------
Staphylococcus	22	19	17	14	------	-----	-----	-----	12	10	8	7	------

Table2. Showing Antibacterial Activity By Disc Diffusion Method

Bacteria	Diameter Of Inhibition Zone(DIZ) in mm												
	Citrus limon				*Citrus maxima*				*Citrus sinensis*				
	100µl	75µl	50µl	25µl	100µl	75µl	50µl	25µl	100µl	75µl	50µl	25µl	
Enterobacteriacea													
E.coli	10	7	4	3	------	-----	-----	-----	4	2	----	-----	------
Salmonella	13	12	10	7	10	8	6	-----	------	-----	-----	-----	------
Proteus	12	10	8	6	------	-----	-----	-----	------	-----	-----	-----	------
Klebsiella	10	9	3	1	------	-----	-----	-----	------	-----	-----	-----	------
Non-Enterobacteriacea													
Vibrio	18	14	12	10	18	10	8	5	-----	-----	----	----	------
Staphylococcus	15	12	10	7	------	-----	-----	-----	10	7	6	2	------

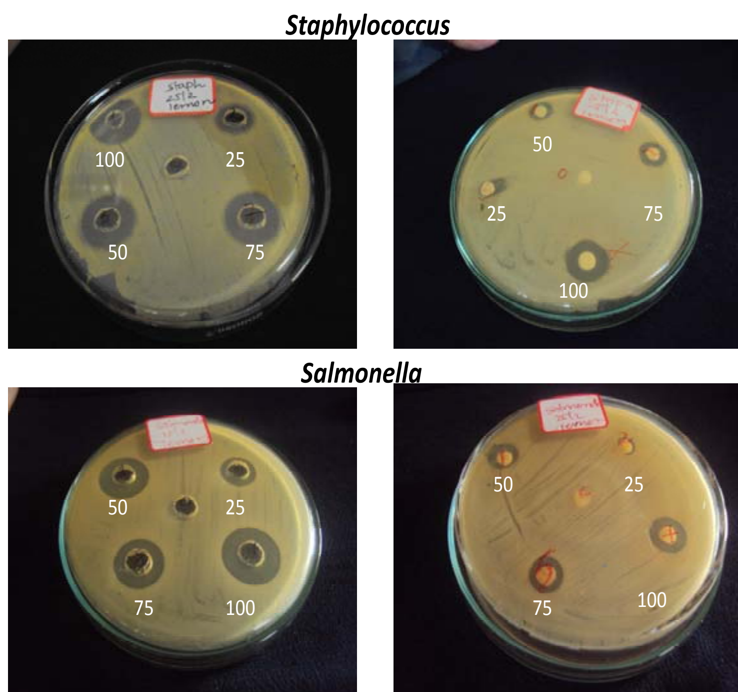

Figure -1:Antibacterial Activity Of Fruit Juice Of *Citrus Limon* By Well Diffusion And Disc Diffusion Methods

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Green Synthesis of Silver nanoparticle using Aerva lanata

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Abstract

This study successfully synthesises silver nanoparticle using ***Aerva lanata*** (***Cherrula***) leaf extract. The leaf extract was screened for phytochemicals. The pH and concentration of plant extract added to silver nitrate concentration was optimised for the best result.

Key Words : Green Synthesis, Silver nanoparticles, *Aerva lanata*

1. Introduction

Among nanoparticles, metal nanoparticles especially silver nanoparticles has attracted considerable attention as a result of their significant applications in the field of in catalysis, sensors and medicine. Over the last decades, silver nanoparticles have found applications in catalysis, optics, electronics and other areas due to their unique size-dependent optical, electrical and magnetic properties. Currently, most of the applications of silver nanoparticles are as antibacterial or antifungal agents in biotechnology and bioengineering, textile engineering, water treatment and silver based consumer products. There is also an effort to incorporate silver nanoparticles in to wide range of medical devices like bone cement, surgical instruments, surgical masks, wound dressing, fluorescent biological labels for important biological markers and molecules in research and diagnosis of diseases. They also find application in the field of drug delivery systems, gene delivery systems in gene therapy, for biological detection of disease causing organisms and diagnosis, detection of proteins, isolation and purification of biological molecules and cells in research, probing of DNA structure, genetic and tissue engineering, in MRI studies, in pharmacokinetic studies etc. (Tiwari A. and SyvÃ M, 2014, Wijnhoven*, et.al.,* 2009).

Silver nanoparticles are nanoparticles of silver i.e. silver particles between 1nm and 100nm in size. Although chemical and

physical methods may successfully produce pure, well-defined nanoparticles, these are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner (Kumar & Yadav, 2009).

Green syntheses of silver nanoparticles when compared to chemical and physical methods are environment friendly and cost effective. In these methods, there is no need to use high pressure, energy, temperature or toxic chemicals. Thus synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining nanoparticles and are preferable over the traditional chemical or mechanical methods for their simplicity, cost-effectiveness, environment friendly nature and reproducibility. (Mittal, *et. al.*, 2016). Synthesis of nanoparticles especially silver using plant extracts has been reported previously. Elumalai *et al.* (2010) reported the synthesis of AgNp using *Euphorbia hirta* leaves, Singha *et al.*, (2010) using *argemone mexicana* leaf extract, Prasanth *et al.* (2011) using medicinal plant extracts and Mallikarjuna *et al.* (2011) using *Ocimum* leaf extract. Synthesis of AgNp from plant extract is carried out by exploiting the reduction capabilities of varied phytochemicals present in them (Amarnath Kanchana *et al.*, 2011)

Here we attempted to find a cost effective & ecofriendly method for the synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of *Aerva lanata* (*Cherrula*). The method involves reducing the silver ions present in the solution of silver nitrate by the different phytochemicals present in the extract followed by capping these nanoparticles. Nanoparticles were characterized using UV-visible absorption spectroscopy using UV-visible spectrophotometer.

2. Materials & Methods

Silver nitrate is used for the synthesis of silver nanoparticles. All glassware have been washed with distilled water and dried in oven before use. The leaf sample of ***Aerva lanata*** (*Cherrula*) was collected locally. The plant samples was cleaned of all the impurities present by washing with normal water followed by distilled water. The sample was dried in shade by keeping overnight in at room temperature.

2.1 Preparation of leaf extract

5 g of dried ***Aerva lanata*** (*Cherrula*) leafs were weighed and transferred to a 250ml beaker, 100ml of double distilled water was added to it and a glass rod was placed inside

the beaker. Then the beaker was placed in water bath, maintained at 80°c for thirty

minutes and cooled. The extract was then filtered using Whatmann No: 1 filter paper into a conical flask. This extract was stored in refrigerator for future use.

2.2 Phytochemical screening of leaf extract

The plant extract was tested for carbohydrates, glycosides, tannins, saponins, phenols, flavonoids, anthrocyanoside, and anthraquinonine.

2.3 Synthesis of silver nanoparticles

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 1.7g of Silver nitrate was weighted accurately in an electrical balance, transferred to the standard flask and made up to 100ml. From this solution, pipette out 50ml $AgNO_3$ solution, and transfer to another 500ml standard flask. Make up to the mark to obtain 1mM $AgNO_3$ solution.

To synthesize silver nanoparticles, the solution of $AgNO_3$ with plant extract in varying ratios and at different pH. To optimize the synthesize AgNP, three different concentrations viz. 1:9, 2:8 and 3:7 were tested. The pH range was varied and the experiment was carried out at pH 6, 8 and 12. The experiment was carried out in conical flask covered with aluminium foil was added to solution of $AgNO_3$ drop wise to prevent the entry of light. Leaf extract slowly with constant shaking .This was placed on shaker for 30 minutes. pH was adjusted by adding necessary quantity of NaOH.

2.4 UV-Visible spectra analysis

The formation of silver nanoparticles was monitored by change in colour of the solution to golden brown. The solution was observed for colour change periodically and allowed to rest in dark overnight if needed. On observing a change in colour, the solution was analyzed with UV spectrophotometer (1800 shimadzu UV spectrophotometer) after diluting a small quantity of sample with distilled water.

3. Results & Conclusion

3.1 Phytochemical screening of leaf extract

Aqueous extracts of the plant ***Aerva lanata*** (*Cherrula*) showed the presence of carbohydrate, glycoside, tannin, saponin, flavonoid, and phenol. Antroquinone and anthocyanin were not found in the extract of ***Aerva lanata*** (*Cherrula*). Table 1 showed the results of phytochemical analysis of the aqueous extracts of ***Aerva lanata*** (*Cherrula*).

Table 1: Phytochemical Analysis of Aqueous Extract of *Aerva lanata*

Sl.No	Name of the phytochemical constituent	Aqueous extract
1	Carbohydrate	+
2	Glycosides	+
3	Tannins	+
4	Saponin	+
5	Phenol	+
6	Flavanoids	+
7	Antroquinon	-
8	Anthocyanin	-

(+) Indicates the presence and the absence of phytoconstituents

3.2 Visual observation and UV -visible Spectral analysis

Noble metals like silver are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR) (Bindhu & Umadevi, 2013). On visual observation it was observed that the colour of the reaction mixture at pH 12 started changing from yellowish brown to reddish brown, indicating the generation of silver nano particals. No colour change was observed in solutions maintained at either pH 6.5 or pH 8. The solution was analysed using UV Visible spectrophotometer. The spectra was scannedbetween 200-700nm at 1nm resolution. A peak specific to the synthesis of silver nanoparticle was observed at λ_{max} 428nm with the absorbance value being 2.7. Control silver nitrate solution neither developed the reddish brown colour nor did they display the characteristic band, indicating that abiotic reduction of silver nitrate did not occur under the test conditions.

The absorption band in visible light region, 350 nm – 550 nm and a plasmon peak at 428 nm is typical for silver nanoparticles. Similar values were previously reported during the biosynthesized AgNPs using medicinal plant *Hovenia dulcis* by Salunke, B K., *et. al.,* in 2016. In their study they observed a peak at 433 nm. An absorbance

peak at 450 nm was also reported by Jain, et al., during synthesis of silver nanoparticles using papaya fruit extract (2009). The surface plasmon resonance of the silver nanoparticles reduced by the Spirulina aqueous extract was obtained at 431 nm in a study by Palanisamy, *et. al.,(*2017).

It can be reasonably concluded that we successfully synthesised silver nanoparticle using extract of ***Aerva lanata*** (*Cherrula*). The optimum pH and concentration for biosynthesis of AgNp were pH 12 and 1:9 concentration.

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Role of capping agent in the synthesis of stable nano ZnO colloid

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Abstract

In this article we present the Synthesis of Stable nano ZnO by colloidal chemical synthesis using a capping agent Poly Vinyl Pyrrolidone (PVP). Absorption spectroscopy is a powerful tool in characterizing nanocrystal colloids and Particle Size is calculated from the shift of absorption edge. Time dependent absorption spectroscopy gives us an idea about Particle Growth over time and the effect of capping in the prevention of growth. It is found that the addition of PVP effectively controls the growth. We found an optimum concentration of PVP to be added in one milli molar ZnO for the effective capping.

***Key words** : Nanocolloid, Absorption spectroscopy, Capping*

1.Introduction

Several approaches have been considered to prepare size-controlled semiconductor nanocrystallites with narrow size distribution. Many of these preparation methods control the growth by the addition of stabilizers or capping agents like Thiols, PVP etc. by restricting the growth space in matrices like Zeolites, reverse micelles, porous silica matrix etc (Vossmeyer, 1994; Yang, 2001; Wang, *1989;* Pillai,1995; Maeda, 1995). Surface-capped CdS colloids have been prepared by different thiols, for example, Henron et al. have shown that CdS nanoclusters of various sizes can be prepared by adjusting the ratio of sulfide to thiophenol during synthesis (Herron, 1990). Yang *et al* (2001) have attempted preparing ZnO quantum dots of different sizes by PVP capping and we have attempted a similar approach.

2.Theory

When quantum dots are excited with energies larger than band gap energy, electrons in the conduction band and holes from the valence bands are excited. Depending on the excitation conditions, the coulomb attraction

between a hole and electron might lead to a bound state, the Wannier Exciton. Just as the hydrogen atom, the exciton states are characterized by a product wave function consisting of a plane wave part for the centre-of-mass motion and hydrogen functions for the e-h relative motion. The characteristic length scale for the relative motion is the exciton Bohr radius, which may be of the order of 1-20 nm, depending on the semiconductor material. Quantum confinement effects arise, as soon as the size of the quantum dot is comparable to this exciton Bohr radius.

In the case of nanocrystallites, the electrons, holes and excitons have limited space to move and their limited motion becomes possible only for definite values of energy. The highest occupies valence band and lowest unoccupied conduction band are shifted to a more negative and positive values respectively resulting in widening of band gap. This leads to a blue shift of absorption band which be observed through optical absorption and transmission studies.

3.Experiment

Stable nano ZnO colloid is prepared by colloidal chemical synthesis using a capping agent Poly Vinyl Pyrrolidone (PVP). One millimolar of zinc acetate is dissolved in isopropyl alcohol (IPA-Merck, HPLC grade) by stirring at 50^0C in the presence of capping agent poly vinyl pyrolidone (PVP-Sisco) (Litty, 2007; Song, 2015). ZnO colloid is formed when it is hydrolysed with sodium hydroxide under ultrasonification for 2 hours. The ZnO colloids are characterized by time dependent optical absorption measurements recorded using a spectrophotometer.

4.Results and Discussions

Absorption spectroscopy is a powerful tool in characterizing nanocrystal colloids. Figure 1 gives the time dependant absorption spectra of uncapped ZnO colloid. The excitonic peak is found to be blue shifted (353 nm) with respect to that of bulk ZnO (395 nm) and this could be attributed to the confinement effects (Luna-Moreno, 2002). As time goes on, there is red shift in absorption edge which indicates the increase in particle size with respect to time.

Figure 2 gives the absorption spectra of PVP capped ZnO for different PVP concentrations. From the above data, we can clearly see how addition of PVP

effectively arrests the growth. We found an optimum concentration of PVP to be added in 1mM ZnO for the effective capping.

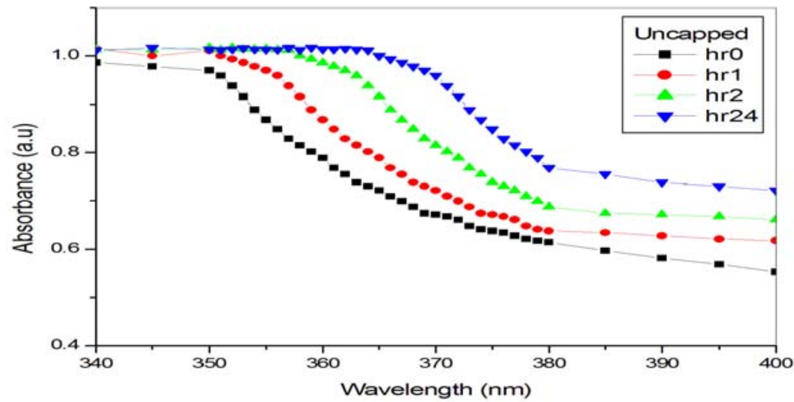

Figure 1: Time dependant absorption spectra of uncapped ZnO colloid

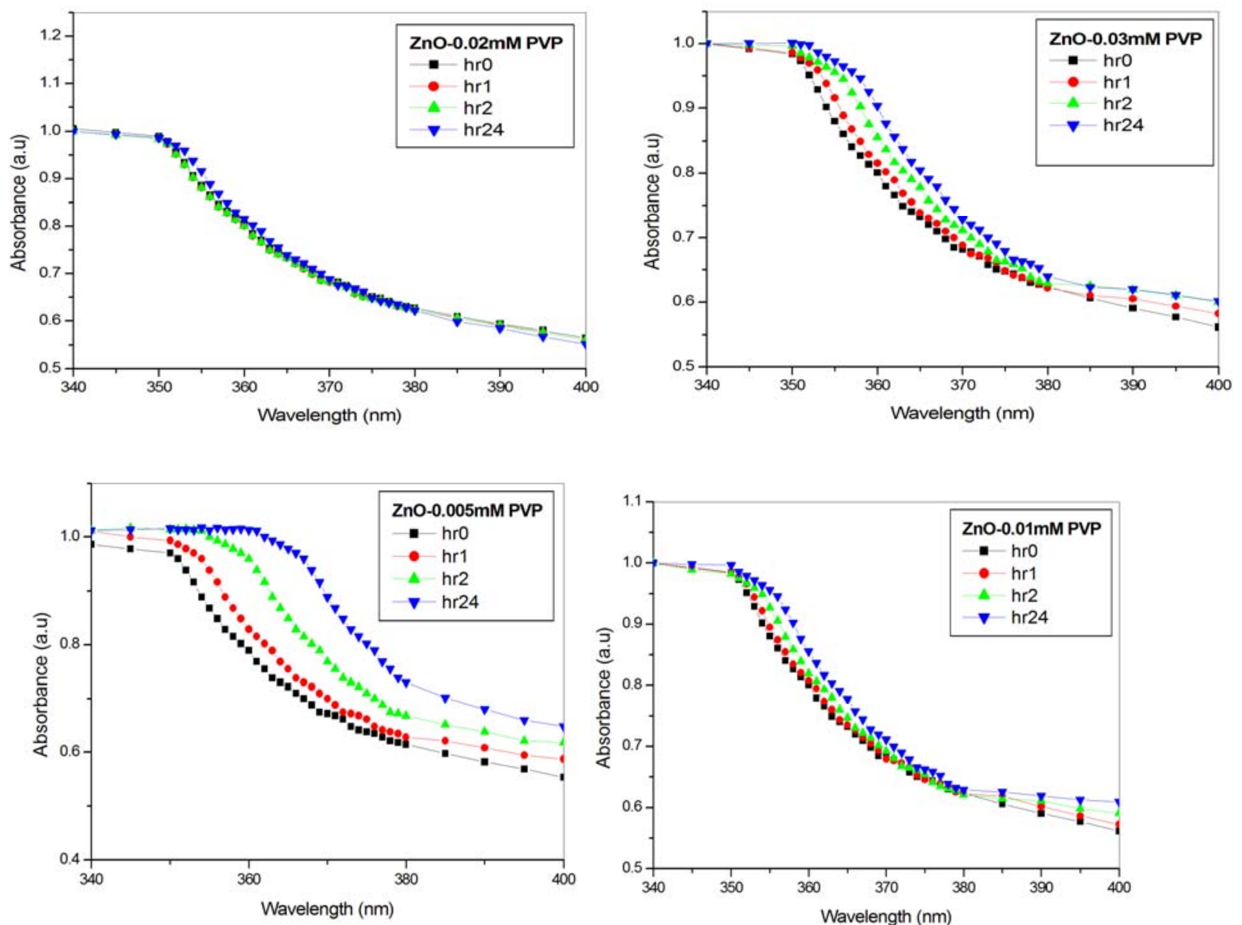

Figure 2: Absorption spectra of PVP capped ZnO for different PVP concentrations

Table 1: Variation of particle size of uncapped/capped ZnO for different PVP concentrations

	Particle Size (nm)				
	Uncapped (1mM ZnO)	*ZnO+ 0.005mM PVP*	*ZnO+ 0.01mM PVP*	*ZnO+ 0.02mM PVP*	*ZnO+ 0.03mM PVP*
0hour	4.4	4.4	4.4	4.4	4.4
1 hour	5.24	4.85	4.5	4.4	4.67
2 hour	7.62	6.35	4.85	4.4	5.25
24 hour	16.37	10.7	5.25	4.67	6.25

The pronounced dependence of the absorption band gap on the size of ZnO nano crystals is used to determine the particle size. The cluster sizes are calculated from the absorption spectra using the analytical formula given by Ranjani *et al* (2004). To get a precise measure of the shift, the first derivative curve of the absorption spectrum is taken and the point of inflection is taken as the cut-off wavelength. From the cut-off wavelength, the corresponding E_g is calculated. The deviation of this from the E_g of bulk ZnO gives ΔE_g and the particle size, d is determined using the equation,

$$\Delta E_g = 100\left(18.1d^2 + 41.4d - 0.8\right)^{-1}.$$

Table 1 gives the variation of particle size of uncapped/capped ZnO for different PVP concentrations.

From the above table, it is clear that uncapped ZnO is unstable since particle size increases with time. It is found that the addition of PVP effectively controls the growth. We changed the concentration of PVP from 0.001mM to 0.03mM and it is found that 0.02Mm PVP capped ZnO is more stable. We found an optimum concentration of PVP to be added in 1mM ZnO for the effective capping.

5.Conclusion

In this article we present the Synthesis of Stable nano ZnO by colloidal chemical synthesis using a capping agent Poly Vinyl Pyrrolidone (PVP). Absorption spectroscopy is a powerful tool in characterizing nanocrystal colloids. Even without sophisticated tools like TEM, one can get an idea

about the size range from the shift of absorption edge. Time dependent absorption spectroscopy gives us an idea about particle growth over time and the effect of capping in the prevention of growth. It is found that the addition of PVP effectively controls the growth. We found an optimum concentration of PVP to be added in 1mM ZnO for the effective capping.

Acknowledgments

Author acknowledge Department of Physics, Vimala College, Department of Chemistry & Zoology, St. Mary's College, Thrissur for experimental facility.

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Preliminary screening, isolation and characterization of cellulolytic bacteria from soil

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Abstract

Microbial cellulases are an important group of enzymes that can have application in various industries such as food processing, laundry industry, leather processing, bioremediation process and in textile industry. Soil is an important source for isolation of microorganisms for novel industrial enzymes production. In this study the cellulase producing bacteria were isolated from soil rich with degrading organic matter. Two potential isolates were identified to be cellulolytic in CMC agar media by Congo Red Method and confirmed by Gram's Iodine Method. These isolates were tentatively identified by some common biochemical tests and microscopical observations. They were then subjected to submerged fermentation for the production of cellulase enzyme and its activity was assayed. They were found to produce around 3 U enzyme at 72 h. Further studies for maximization the enzyme production followed by its purification, characterization and applications are to be undertaken.

Keywords: *Cellulase, CMC agar, submerged fermentation, Congo Red, Gram's Iodine.*

1. Introduction

Cellulose is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bio resource produced in the biosphere. Biotechnological conversion of cellulosic biomass is potentially a sustainable approach to develop novel bioprocesses and products. Cellulose is commonly degraded by an enzyme called cellulase which hydrolyses the β-1,4- glycosidic bonds in the polymer to release glucose units. Cellulases are inducible enzymes synthesized by large diversity of microorganisms including both fungi and

bacteria during their growth on cellulosic materials. These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. The genera of *Clostridium, Cellulomonas, Aspergillus Trichoderma* and *Thermomonaspora* are the most extensively studied cellulase producers.

Microbial cellulases have become the focal biocatalysts due to their complex nature and widespread industrial applications.

Cellulose containing wastes may be agricultural, urban, or industrial in origin; sewage sludge might also be considered a source of cellulose since its cellulosic content provides the carbon needed for methane production in the anaerobic digestion of sludge. Agricultural wastes include crop residue, animal excreta and crop-processing wastes, slashing generated in logging, sawdust formed in timber production, and wood products in forestry originated activities.

Cellulase hydrolysis of the β-1,4-glucosidic linkages yield glucose, cellobiose and cello-oligosaccharides as the primary products. Cellulases are the most extensively studied multiple enzyme complex comprising of endoglucanases (EG), cellobiohydrolases (CBH), β-glucosidases (BGL). Endoglucanases produces nicks in the cellulose polymer exposing reducing and non-reducing ends, cellobiohydrolases acts upon these reducing and non-reducing ends to liberate cello-oligosaccharides and cellobiose units and β-glucosidase cleaves the cellobiose to liberate glucose, thereby completing the hydrolysis. The complete cellulase system comprises of CBH, EG and BGL components which act synergistically to convert crystalline cellulose to glucose.

Cellulases were initially investigated several decades back for the bioconversion of biomass which gave way to research in the industrial applications of the enzyme in animal feed, food, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry. Cellulases are currently the third largest industrial enzymes worldwide. Cellulase due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol (Ekperigin, 2007; Vaithanomsat *et al*, 2009), agricultural and plant waste management (Lu *et al*,

2004; Mswaka, 1998); chiral separation and ligand binding studies (Nutt, 1998). The present study was on the screening of cellulase producing isolates from soil, followed by gram staining and different biochemical tests in an attempt for preliminary identification. Also, fermentative production of cellulase enzyme was carried out to quantify the enzyme produced by the bacterial strains.

2. Materials and Methods

2.1 Sample Collection for Screening and Isolation of Cellulolytic Bacteria

Cellulase producing bacteria were isolated from soils collected from different regions in the college campus. The soil samples were serially diluted and spread plate technique employed using CMC selective agar media containing 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% carboxy methyl cellulose (CMC) sodium salt, 0.02% peptone and 1.7% agar. The plates were incubated at 37° C for 24-48 hours followed by visualization of cellulose hydrolysis zone.

2.2 Detection of Cellulolytic Bacteria – Congo Red Method

To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15min and washed with 1M NaCl (Apun et al., 2000). The diameter of the clear zone around colonies on CMC agar was measured which indicates the cellulose activity of the organisms. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulose activity producer. The largest ratio was assumed to contain the highest activity.

2.3 Detection of Cellulolytic Bacteria - Gram's Iodine Method

The isolates with cellulose hydrolysis zone detected by Congo red staining were spot plated onto CMC agar and incubated at 37°C for 3 days. The plates were then flooded with Gram's Iodine solution for 3-5 minutes according to Kasana *et al*., (2008) and zone of clearance around the colony observed.

2.4 Identification of CDB isolates

The cellulose degrading bacterial isolates were presumptively identified by means of morphological examination and some

biochemical characterizations. The parameters investigated included colony morphology, Grams stain, microscopic observations like morphology and arrangement of cells, motility and biochemical tests including catalase production, IMViC reaction, Catalase, Oxidase tests etc.

2.5 Fermentative Production of Cellulase enzyme

Production medium contained (g/L) glucose 0.5 gm, peptone 0.75 gm, $FeSO_4$ 0.01 gm, $KH_2PO_4$0.5 gm, and $MgSO_4$ 0.5 gm. Fifty millilitres of medium were taken in a 100mL conical flask. The flasks were sterilized in autoclave at 121^oC for 15 min, and after cooling, the flask was inoculated with overnight grown bacterial culture. The inoculated medium was incubated at 37^oC in shaker incubator for 24-72 h. At the end of the fermentation period, the culture medium was filtered to obtain the crude extract, which served as enzyme source.

2.6 Enzyme Assay

Cellulase activity was measured following the method of Miller [18]. The reaction mixture composed of crude enzyme solution and carboxymethyl cellulose (CMC) in 50 mM citrate buffer which was incubated at 45^oC in a water bath for 30 min. The reaction was terminated by adding 3mL of DNS reagent. The colour was then developed by boiling the mixture for 10min followed by rapid cooling. OD of samples was measured at 540nm against a blank. Cellulase production was estimated by using glucose calibration curve. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1mol of glucose per minute under standard assay conditions.

3. Results and Discussion

Cellulose is the main building block of plants and form major fraction of organic carbon in soil. Microorganisms, which live in soil, are accountable for recycling of this organic carbon. Degradation of cellulosic materials is a complex process and requires participation of microbial cellulolytic enzymes. Habitats where these substrates are present are the best sources for isolation of cellulolytic microorganisms. The study aimed at isolating and identifying a potential source of cellulase enzyme initiated with the screening of different environment

samples in CMC agar media to qualitatively identify the enzyme source. This was followed by the identification of the major producer using various morphological, staining and biochemical characteristics, submerged fermentative production of the enzyme and estimation of its cellulolytic activity.

3.1 Detection of Cellulolytic Bacteria

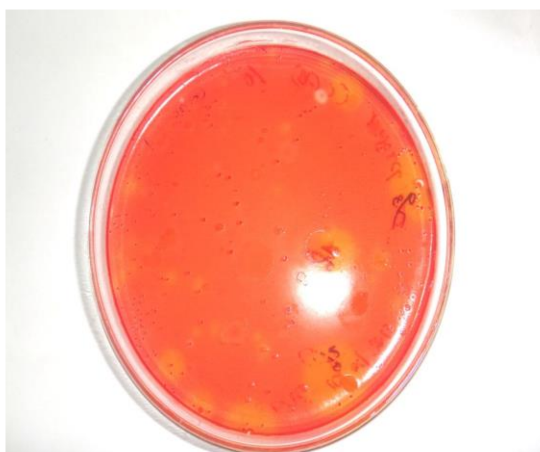
Cellulose degrading bacteria were isolated from soil samples using CMC agar medium and Congo red staining. The use of Congo red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discoloration of Congo red were taken as positive cellulose degrading bacterial colonies.

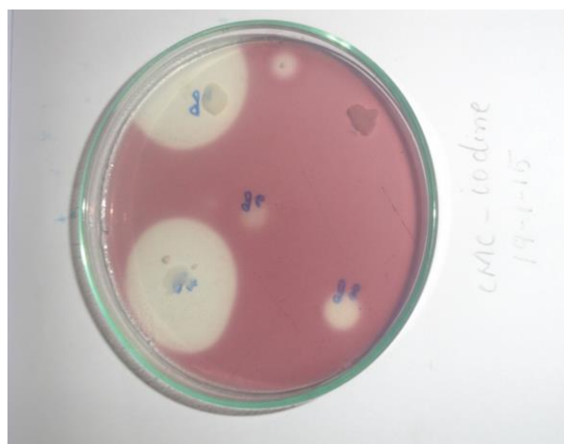
Ten isolates showed the development of clear zones with Congo red in CMC agar medium and the clearing zone ranged from 0.5 cm to 1.9 cm, demonstrating that the isolates have the ability to degrade the carboxymethylcellulose and indicating the high probability of cellulase production.

These results were confirmed with staining using Gram's Iodine also in CMC agar plates. Two isolates, G_1 and G_3 with reasonable zones of hydrolysis were selected for further studies.

Table 1: Detection of Cellulolytic Bacteria

Sl No	Isolate	Zone of hydrolysis (in cm)	
		Congo Red Staining	Gram's Iodine
1	G_1	1.9	2.3
2	G_3	2	2.5





Congo Red Method **Gram's Iodine Method**

Figure 3: Detection of Cellulolytic Bacteria

3.2 Identification of CDB isolates

The results of staining and biochemical examinations are summarized in Table 2.

From the observations G_1 is presumably identified as Microbacter and G_3 is to be further characterized.

Table 2: Presumptive Microscopic and biochemical identification of G_1 and G_3

Sl No	Test	G_1	G_3
1	Gram Staining	Gram +ve rods	Gram –ve cocci
2	Motility	Motile	Non motile
3	Catalase	Negative	Positive
4	Oxidase	Negative	Negative
5	Sugar Fermentation a) Maltose b) Lactose c) Glucose d) Sucrose e) Mannitol	 Acid Acid Acid No change Acid	 Acid Acid Acid No change No change
6	IMViC a) Indole Production b) Methyl Red Test c) Voges Proskaeur d) Citrate Utilization	 - + - -	 - + - -

3.3 Fermentative Production of Cellulase enzyme

The isolates G_1 and G_3 from soil which gave a reasonable zone of hydrolysis in the CMC agar plates were inoculated into 100ml production medium for a period of 72h with routine sampling done at 24 h interval. The culture filtrates were subjected to enzyme activity determination which was calculated against a glucose standard curve. Cellulase system consist of 1,4-β-endoglucanase, 1,4-β-exoglucanase, and β-glucosidase which synergistically do complete hydrolysis of cellulose to glucose. DNS reagent was used to stop the enzymatic **r**eaction, and the reaction product measured as a result of the reaction between glucose and the DNS reagent. One unit of enzyme activity was defined as the amount of enzyme required to release the 1μmole of glucose/min under standard conditions.

Table 3 Cellulase activity during submerged fermentation

Time (h)	**Cellulase activity ($U\ ml^{-1}$)**	
48	1.4	3.13
72	1.62	3.6

The activity was found to increase with time and the maximum was recorded at 72 h. Both G_1 and G_3 showed similar kind of enzyme activity with a yield of around 3 Units at the end of 72 h. Further investigations are required to record the enzymatic activity as a function of time and to optimize the conditions for maximization the enzyme production.

4. Conclusions

Microbial cellulases are an important group of enzymes that can have application in various industries such as food processing, laundry industry, leather processing, bioremediation process and in textile industry. Cellulases are widespread in nature; microbes serve as a preferred source of these enzymes because of their

extreme habitat variability, rapid growth, the limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications. The future may hold great prospects for lignocellulosic biofuel; by combining our knowledge of excellent cellulolytic and hemi cellulolytic with technologies such as directed evolution and co-culture, the future of lignocellulolytic biofuel looks potentially feasible.

Soil is an important source for isolation of microorganisms for novel industrial enzymes production. Hence, in this present study also the cellulase producing bacteria were attempted to be isolated from soil. Two potential isolates designated as G_1 and G_3 were found to be cellulolytic in CMC agar media and were tentatively identified by some common biochemical tests and microscopical observations. They were further subjected to submerged fermentation for the production of cellulase enzyme and its activity was assayed. They were found to produce around 3 U enzyme at 72 h.

Further studies like determining the time course of enzymatic activity and validating other parameters for maximization the enzyme production followed by its purification, characterization, study of optimal conditions of activity and its stability followed by applications are to be undertaken.

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Synthesis of Two Novel Nnn-Donor Schiff Bases And Their Characterization

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Abstract

Two new Schiff bases, Quinoxaline-2-carboxalidine-2-aminopyrimidine and Quinoxaline-2-carboxalidine-3-aminopyridine, have been synthesized by condensation from quinoxaline-2-carboxaldehyde and 2-aminopyrimidine / 3-aminopyridine. The Schiff bases are characterized by elemental analysis, FT-IR, UV-Vis, TG, and NMR spectroscopy. Spectroscopic characterization clearly reveals the tridentate NNN-donor behaviour of the Schiff base.

Keywords: *Schiff base, quinoxaline-2-carboxaldehyde, 2-aminopyrimidine, 3-aminopyridine*

1. Introduction

Multidentate ligands are widely used in coordination chemistry, since they found useful in the assembly of new frameworks with attractive properties. Among these ligands the transition metal complexes containing nitrogen donor atoms are of considerable interest in inorganic and biomimetic chemistry due to their possible application in catalysis, medicine and material science (Gupta and Sutar, 2008). These ligands are of interest because of their ability to form transition metal complexes which have varying configurations, structural lability and sensitivity to molecular environments (Cozzi, 2004). They have proven to be effective in constructing supramolecular architectures such as coordination polymers and helical assemblies. Quinoxalines with other heterocyclic amines groups are of particular interest

due to their wide applications (Sharma and Varshney, 1991) (Cheeseman, 1982).

They can form complexes either in bidentate NN form or in tridentate NNN form. The synthesis and characterization of Schiff bases derived from quinoxaline-2-carboxaldehyde with various amines and their complexes were previously reported (Arun et al, 2009)(Sebastian et al, 2010). The objective of current work is to prepare new tridentate NNN donor Schiff base from quinoxaline-2-carboxaldehyde and 2-aminopyrimidine / 3-aminopyridine.

The Schiff base is synthesized by condensation of aldehyde and amine. The Schiff base is an NNN donor and it can act as a monobasic or dibasic tridentate ligand due to the possibility of different coordinating nature of metal ion.

2. Materials and methods

2.1 Materials and physical measurements

2-aminopyrimidine (Aldrich) and 3-aminopyridine (Aldrich) are used as supplied. The preparation of quinoxaline–2–carboxaldehyde is carried out as reported in the literature (Mayadevi et al, 2003). Elemental analyses of the ligands were done on a Vario EL III CHNS analyzer. The IR spectra were recorded on a JASCO FTIR–4100 spectrometer using KBr pellets in the range 400–4000 cm^{-1}. The electronic spectra of the ligands were recorded on a Thermoelectron Nicolet evolution 300 UV-Vis spectrophotometer.

2.2 Synthesis of Schiff base Quinoxaline-2-carboxalidine-2-aminopyrimidine (Abbreviated as L1)

Take 1mmol of quinoxaldehyde (1.5816g) in 50ml acetonitrile. To this add 1mmol of 2-aminopyrimidine (0.951g) in 30ml acetonitrile. The mixture is refluxed in a 100ml R.B. for 6 hours. Reduce the volume, cool to room temperature. The crystals formed are filtered out washed with water to remove the traces of unreacted amine, followed by petroleum ether. An orange colored powder is obtained finally (Figure 1). Yield: ~65%.

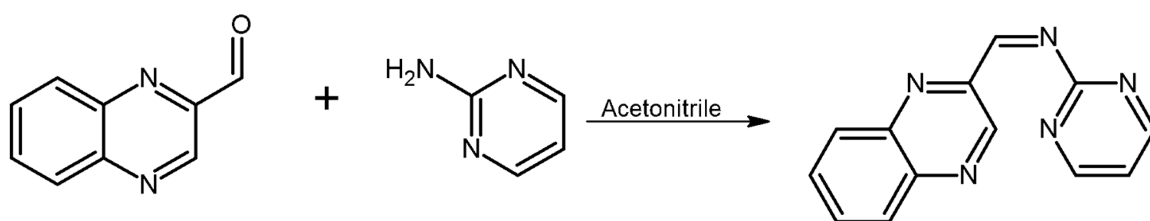

Figure 1: Synthesis of Quinoxaline-2-carboxalidine-2-aminopyrimidine

2.3 Synthesis of the Schiff base Quinoxaline-2-carboxalidine-3-aminopyridine (Abbreviated as L2)

An ethanolic solution of (20ml) 3-aminopyridine (10mmol, 0.9412g) and quinoxaline-2-carboxaldehyde in 30ml ethanol (10mmol, 1.5816) was boiled under reflux for 3 hours. The volume of the solution was reduced to one by third. The solvent was allowed to evaporate. It was then recrystallised from methanol (Figure 2).

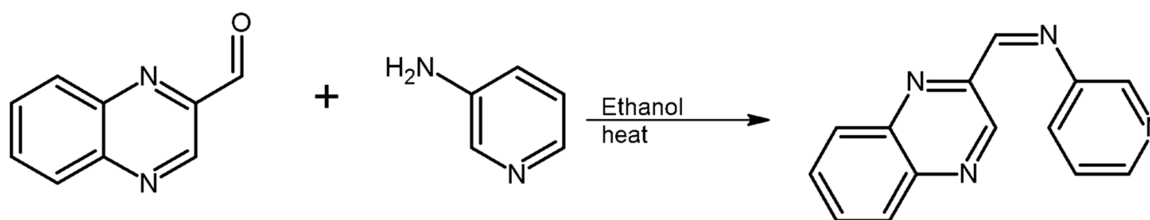

Fig: 2- Synthesis of Quinoxaline-2-carboxalidine-3-aminopyridine

3. Results and discussion

The preparation of ligands L1 and L2 (figure 1 and 2) were carried out in acetonitrile/ethanol and the yield is found to be good. Elemental analysis of the ligands is given in Table 1 and it is found that the found and calculated values are in good agreement.

4.2.1. FT-IR Analysis

The assignments of IR spectral bands most useful in establishing the structural identity of the ligands are listed in Table 2. The azomethine bands ν(C=N) appear

at 1600 - 1700 cm^{-1}, is in agreement with earlier reports. The quinoxaline ring C=N stretching was found near 1580 cm^{-1}. In

ligand L2, the in-plane and out-of-plane deformation vibrations characteristic of pyridyl ring are observed respectively in the range 613-622 and 401-421cm^{-1} (Bermejo, 1999). Pyrimidines are characterized with strong absorption in the range 1600-1500 cm^{-1} due to the C=C and C=N ring stretching vibrations. Usually 2-substituted pyrimidines have three medium to strong absorption bands in the range 650-630, 580-475 and 515-440 cm^{-1} (George, 2001). The infrared spectrum of compound L1 contains three bands in the above said range indicates pyrimidyl moiety in the Schiff base.

Table 1: Analytical data of L1 and L2

Compound	Stoichiometry	Colour	Yield (%)	Analytical data. Found (calculated)%		
				C	N	H
L1	$C_{13}H_9N_5$	Violet	86	65.83 (66.37)	29.25 (29.77)	4.36 (3.85)
L2	$C_{14}H_{10}N_4$	Yellow	81	71.55 (71.78)	23.21 (23.92)	4.36 (4.30)

4.2.1. FT-IR Analysis

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Table 2: IR spectral data of L1 and L2

Assignments	**ν (C=N)#**	**ν (C=N)***	**ν $(py)_{ip}$**	**ν $(py)_{op}$**	**ν $(pm)_1$**	**ν $(pm)_2$**	**ν $(pm)_3$**
L1	1698	1587	-	-	621	477	441
L2	1617	1574	624	403	-	-	-

 # azomethine, * quinoxaline, py-pyridyl, pm-pyrimidyl

4.2.2. UV-Vis Spectral Analysis

The tentative assignments of the significant electronic spectral bands of ligands are presented in Table 3. The electronic spectra of L1 and L2 in DMF solution show the following intraligand absorption maxima: Two bands corresponding to π— π* transitions of the pyridyl ring, pyrimidyl ring, benzene ring and imine function of the schiff bases are observed in the range 41840-43290 cm^{-1} and 33560- 38760 cm^{-1}; the n— π* transitions of the pyridyl ring and imine function are observed in the range 29500-30960 cm^{-1} (Neelakandan et al, 2008).

Table 3: Electronic spectral data (in DMF)

Compound	**Absorption maxima**	**Tentative assignments**

L1	42716, 44562	π— π*
L1	29530	n— π*
L2	42760, 43190	π— π*
	32511	n— π*

Table 4: ^{1}H NMR Spectroscopic data of L1 and L2

Compound	Chemical shift, δ (ppm)	Assignment
L1	8.78 6.74-8.22	(s, 1H, CH azomethine) (m, 8H, Ar)
L2	8.99 7.06-8.26	(s, 2H, CH azomethine) (m, 14H, Ar)

4.2.3. NMR Spectral Analysis

The ^{1}H NMR spectrum of L1 and L2 shows signals at 8.08 and 8.99 ppm corresponding to azomethine CH protons respectively. Aromatic protons appear as a multiplet at 6.74-8.26 ppm. The detailed result of NMR spectral analysis is listed in table 4. The assignments are in agreement with the values already reported.

4. Conclusions

Synthesis and characterization of two novel Schiff bases Quinoxaline-2-carboxalidine-3-aminopyridine and Quinoxaline-2-carboxalidine-2-aminopyrimidine have been carried out. Schiff bases are prepared by condensation between aldehyde and primary amine. Aldehyde selected for current work is quinoxaline-2-carboxaldehyde which is a heterocyclic aldehyde. The primary amines used were 2-aminopyrimidine and 3-aminopyridine. Ligands are characterized by elemental analysis, infrared, UV-Vis and H^1 NMR spectral studies. Elemental analysis of ligands shows that the found and calculated values are in good agreement. An infrared spectrum of Schiff bases gives a medium band in the range 1600-1650 cm^{-1} characterized azomethine stretching of Schiff base. Spectroscopic characterizations clearly revealed the

bidentate NN or tridentate NNN-donor behaviour of the Schiff base.

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Antioxidant Potential of Ethanolic and Hot Water Extracts of *Pleurotus Sajor-Caju*

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Abstract

Pleurotus species are commonly called oyster or paddy straw mushrooms. The genus *Pleurotus* comprises some most popular edible mushrooms due to their favorable organoleptic and medicinal properties, vigorous growth and undemanding cultivation techniques. In the present investigation, *Pleurotus sajor-caju* fruiting bodies were cultivated on coir pith, ethanolic and hot water extracts were prepared from it. The yield of the mushroom was found to be 24.33%. The yield of the ethanolic and hot waterextract was found to be 10.1 and 7% respectively. The DPPH free radical scavenging activity of the ethanolic and hot water extracts of the fruiting body was studied. Both extracts showed significant activity in a dose dependent manner. At the maximum tested dose, ethanolic extract showed 90.11% activity and the hot water extract showed 90.87% activity. IC_{50} value of ethanolic and hot water extracts were found to be 29 µg and 30 µg, respectively. Present study revealed the significant antioxidant activity of the ethanolic as well as hot water extract from the fruiting body of the edible mushroom *Pleurotus sajor-caju*. The cultivation of the mushroom and isolation of active principles from the mushroom is simple, economical and can be carried out with minimal effort. In this context, the active principles from *Pleurotus sajor-caju* can be a suitable candidate as antioxidant agent.

Key words: *Mushrooms, antioxidant, Pleurotus sojor-caju, free radicals*

1. Introduction

The use of mushrooms as a food item is probably as old as civilization. Mushrooms contain many biologically active compounds with high utility values such as polysaccharides, oligosaccharides, lipids, terpenoids and steroids in the fruiting bodies and/or mycelium. The longest tradition of using edible mushrooms for medicinal purposes is in China. Mushrooms represent the major as yet largely untapped source of powerful new pharmaceutical products. Of approximately 10,000 species of mushrooms, 2000 are safe and 300 are of

pharmacological significance (Hobbs,1995; Miles and Chang,1997). Mushrooms have been reported to have significant pharmacological effects and physiological properties such as bio regulation, maintenance of homeostasis, regulation if biorhythm, cure if several diseases like cancer, cerebral stroke, and heart diseases. They have also demonstrated to have effective substances to decrease blood cholesterol, hypolipidemic, antithrombotic, hypotensive, and other applications (Wasser, 1999).

The protein bound polysaccharides isolated from the mushrooms have been used as an immunmodulatory agent in the treatment of cancer in Asia over 30 years. A large number of mushroom-derived compounds, both cellular components and secondary metabolites, have been shown to affect the immune system and could be used to treat a variety of diseased states. (Wasser, 1999). Unique anti-cancer preparations were developed from mushrooms such as Lentinan from *Lentinus edodes*, Krestin from *Trametes versicolor*, and Schizophyllan from *Schizophyllum commune* (Mizuno, 1996 and 1999). Polysachharides from mushrooms do not attack cancer cells directly but produce their anti-tumour effects by activating different immune responses in the host.

Pleurotus species are commonly called oyster or paddy straw mushrooms. The genus *Pleurotus* comprises some most popular edible mushrooms due to their favorable organoleptic and medicinal properties, vigorous growth and undemanding cultivation techniques. It can be cultivated on log and a wide variety of agroforestry by-products, weeds, and wastes for the production of food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification. Many different techniques and substrates have been successfully utilized for mushroom cultivation and biomass production by means of solid state and submerged liquid fermentation. *Pleurotus* species are now widely consumed as food in East and increasingly in the West (Breene, 1990; Chang, 1993).

A number of polysaccharides or exopolysaccharides (EPS) from cell wall, fruiting body and culture filtrate of *Pleurotus sajor-caju* have been reported with some potential pharmaceutical properties. Among these polymers, homo

and heteroglucans, with β-(1→3), β-(1→4) and β-(1→6) glycosidic linkages, are supposed to play a key role in some health aspects of mushrooms (Manzi & Pizzoferrato, 2000).

In that two decades these have been an explosive interest in the role of oxygen free radicals, more generally known as 'Reactive Oxygen Species' (ROS) and ' Reactive Nitrogen Species'(RNS).A free radical is an atom or molecule that has a single unpaired electron in its outer shell. They are highly reactive compounds that are produced in the body during normal metabolic functions or introduced from environment. They are inherently unstable, since they contain extra energy. To reduce their energy load, free radicals react with the certain chemicals in the body and in the process interfere with the normal functions of cells. The oxidants or free radicals have very short life and damaging activity towards macromolecules like proteins, DNA and lipids.

Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. In recent years, there has been an increased interest in the application of antioxidant to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. The effect of reactive oxygen species and nitrogen species is balanced by antioxidants as well as by antioxidant enzymes. Such antioxidant defenses are extremely important as they represent the direct removal of free radicals (prooxidants).Enzymatic antioxidants involve super oxide dismutase(SOD), catalase (CAT), and glutathione peroxidase(Gpx) (Maron et al., 1979). Non enzymatic antioxidants involve vitamin C, carotenoids thiol antioxidants (glutathione, thioredoxin, and lipoic acid);natural flavonoids; melatonin, a hormonal product of pineal gland; and other compounds (Mc Call & Frei, 1999). Reducing exposure to free radicals and increasing intake of antioxidant nutrient have the potential to reduce the risk of free radicals related health problems. Over the past several years , nutritional antioxidants have attracted considerable interest as potential treatment for a wide variety of disease states, including cancer,

atherosclerosis, chronic diseases and aging .Studies have shown that dietary supplementation with extra antioxidants can reduce the risk of developing cancer.

In the present investigation, *Pleurotus sajor-caju* fruiting bodies were cultivated on coir pith, ethanolic and hot water extracts were prepared from it. An attempt was made to evaluate the anti-oxidant properties of the extracts prepared from *P.sajor-caju.*

2. Materials and Methods

2.1. Pleurotus sajor-caju : The spawn culture of *P. sajor-caju* was obtained from Kerala Agricultural University, Thrissur ,Kerala, India.

2.2. Cultivation of Pleurotus sajor-caju on coir pith

Spawn culture of *Pleurotus sajor-caju* was obtained from Kerala Agricultural University, Thrissur, Kerala , India. Coir pith was soaked overnight in water. It was drained until approximately 60% of water content retained and sterilized. Sterilized coir pith was transferred to long sized well aerated polythene bags. It was then layered with the *P. sajor-caju* spawn culture. Again a layer of sterilized coir pith was placed following a layer of spawn culture. This was repeated till the mouth part of polythene bag. Mouth of the bag was tied and kept in a raised platform in a well-ventilated cropping room for about 15 days. At this time when mycelia became visible inside the polythene bag over the surface of coir pith , the bags were cut and gently removed. Then the coir pith formed a compact mass which was watered daily in order to maintain the moisture. Within 3 days, first flash of the fruiting body became apparent and it was harvested when young. Subsequent harvesting was done as the fruiting bodies are mature.

2.3. Preparation of Ethanolic extract of Pleurotus sajor-caju

The fruiting body of the mushroom was washed thoroughly and cut into small pieces. It was then dried at 40-50°C in hot air oven. The powdered material was defatted with petroleum ether using soxhlet apparatus for 8-10 hours. Then, the powdered material was subjected to 70% ethanolic extraction at 50-70° C for 9-10 hours using Soxhlet apparatus. The extracts were pooled together and evaporated to dryness.

2.4. Preparation of Hot water extract of Pleurotus sajor-caju

The fruiting body of the mushroom was washed thoroughly and cut into small pieces. It was then dried at 40-50ºC in hot air oven. The powdered material was added to a little water in a beaker. The beaker was placed in a boiling water bath for 8-9 hours. The mixture was then filtered and extract obtained was evaporated to dryness.

2.5. DPPH free radical scavenging activity (Antioxidant activity)

Free radical scavenging activity of the extracts were studied using the method of Aquino *et al*., 2001. DPPH in its radical form has absorption at 515nm , which disappeared on reduction by an antioxidant compound. Various concentrations of drug (50-2000µg) combined with 1ml of freshly prepared DPPH reagent solution. The samples were kept for 20 minutes in dark and the decrease in absorbance was measured at 515 nm. The percentage inhibition activity was measured using the formula; **% inhibition = C-T/C×100;** where C was the absorbance of control and T was the absorbance of treated/ standard. IC_{50} value was calculated by plotting concentration of the drug against percent activity.

3. Results

3.1. Cultivation of P. sajor-caju on coir pith

After 14 days of incubation, there was uniform growth of fruiting body along the sides of the coir pith (Figure 1). 608.30 grams of fruiting body were harvested from 2500 grams of coir pith. The yield of the mushroom was found to be 24.33%.

3.2. Preparation of Ethanolic extract from the fruiting body of P. sajor-caju

Extraction was done thrice, in batches for 9-10 hours. The extracts were pooled together and evaporated to dryness. Dark brown colored powder was obtained which was maintained to 4°C, till use. The yield of the extract was found to be 10.1%.

3.3. Preparation of hot water extract from the fruiting body of P. sajor-caju

Extraction was done thrice, in batches for 8-9 hours in boiling water bath and filtered, evaporated to dryness. Dark brown colored filtrate was obtained. The yield of the extract was found to be 7%.

3.4. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the ethanolic and hot water extracts of

the fruiting body was studied. Both extracts showed significant activity in a dose dependent manner (Figure 2 and 3). The ethanolic extract showed 90.11% activity at the maximum tested dose of 2000µg. The hot water extract of *P. sajor-caju* showed 90.87% activity at the maximum tested dose of 2000µg. IC_{50} value of ethanolic and hot water extracts were found to be 29 µg and 30 µg, respectively.

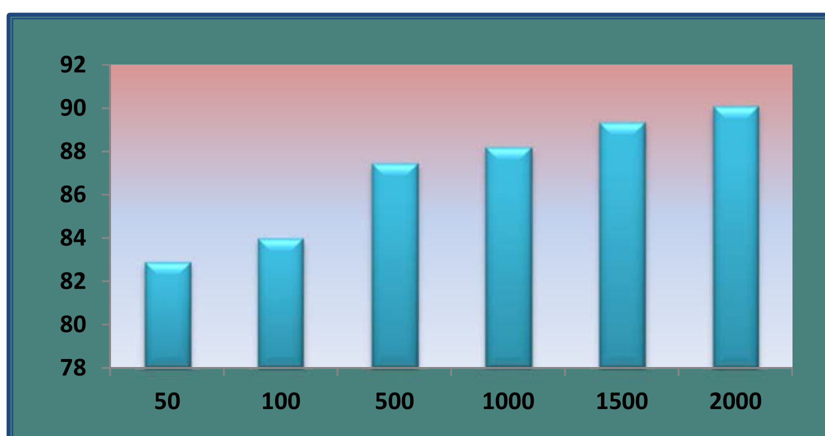

Figure 1: Antioxidant activity of ethanolic extract of *P. sajor-caju* by DPPH free radical scavenging assay.

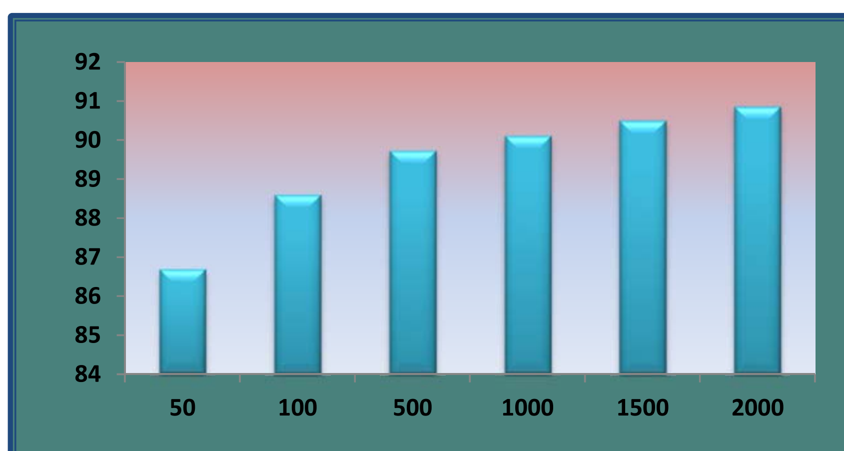

Figure 2: Antioxidant activity of hot water extract of *P. sajor-caju* by DPPH free radical scavenging assay.

Figure 1: Stages of Mushroom Cultivation

4. Discussion

Mushrooms have a great potential for the production of useful bioactive metabolites and they are a prolific resource for drugs. The spectrum of pharmacological activities of mushrooms is fascinating. Discovery and evaluation of metabolites from the various medicinal mushrooms as new safe compounds for treatment of various diseases has become a hot research spot.

Different mushroom species possess different constituents and in different concentration, which account for the different biological activities. Mushrooms usually contain a wide variety of free radical scavenging molecules, such as

polysaccharides, proteins and polyphenols. The mechanism underlying the free radical scavenging activity exerted by these molecules is still not fully understood. Tsiapali etal.,(2001) speculated that the abstraction of anomeric hydrogen from monosaccharide was the reason for the free radical scavenging ability of mushroom polysaccharides. Polysaccharides enhanced antioxidant activity over monosaccharides because polysaccharides exhibited greater ease of abstraction of the hydrogen from one of the internal monosaccharide units.

In the present investigation, *Pleurotus sajor –caju* was cultivated on the agro waste coir pith. Fruiting bodies were produced approximately 14 days after the bagging. Antioxidant properties of the ethanolic as well as hot water extract prepared from this mushroom were studied. Present study revealed profound antioxidant activity of ethanolic and hot water extracts of *Pleurotus sajor –caju* by DPPH free radical scavenging activity. Both drugs showed significant activity in a dose dependent manner. Presence of the active principles like polysaccharides and proteins in these extracts may be responsible for the anti-oxidant properties. However, this needs further confirmation by adequate experimental evidences.

5. Conclusion

Present study revealed the significant antioxidant activity of the ethanolic as well as hot water extract from the fruiting body of the edible mushroom *Pleurotus sajor-caju.* The cultivation of the mushroom and isolation of active principles from the mushroom is simple, economical and can be carried out with minimal effort. In this context, the active principles from *Pleurotus sajor-caju* can be a suitable candidate as antioxidant agent. However, further research is necessary to elucidate the structure and mechanism of action of these active components.

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A comparison on probiotic characteristics of beta-galactosidase producers from curd and raw milk: A review

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Abstract

Probiotics are defined as microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance. The aim of probiotic approach is to repair the deficiencies in the micro flora and restore the animal's resistance to disease. Lactose intolerance or inability to hydrolyze lactose is a problem prevalent in more than half of world's population. Intolerance is mainly occurs due to the absence of galactosidase (rather the presence of a nonfunctional galactosidase) an enzyme associated with the metabolism of lactose. The deficiency of functional galactosidase in our body can be cured by the use of its probiotic producers in the form of easily available dietary sources. Microorganisms are the important sources of many such enzymes, therefore efforts are now increasing to produce these enzymes by microbial fermentation. Selection and isolation of the right organism from a right source influence high yields of desirable enzymes. Major such dietary sources of beta-gal are raw milk and curd. Among these two sources, which one can act as the most beneficial and promising source of beta - gal is to be compared and confirmed for better commercial production the enzyme. This review focuses on the comparison of these two different sources, yielding different probiotic producers of a single enzyme.

Key words: Beta-galactosidase, Lactose intolerance, galacto – oligosaccharides, Ortho Nitro Phenyl beta-D-Galactoside

1. Introduction

Beta-galactosidase (beta-gal) catalyzes the hydrolysis of Beta-galactosides (carbohydrates containing galactose) into monosaccharides through the breaking of a glycosidic bond. Substrates of this enzyme include lactose, lactosylceramides, GM 1 and various glycoproteins (1). This enzyme is also well known as 'lactase'. The lactose hydrolyzation converts lactose in to galactose and

glucose which will proceed in to glycolysis. Substrate analogue galactosides such as PETG (poly ethylene terepthalate glycol) and IPTG (Isopropyl Beta -D-1-Thio Galactopyranoside) will bind in the shallow site (non-productive binding) when the enzyme is in open confirmation (2).

1.1 *Microbial Sources of Beta-galactosidase*

The advantages of microbial Sources over the other include easy handling, higher multiplication rate and high yield (3). Among the microbial sources, bacterial sources are preferred due to ease of fermentation, high enzyme activity and good stability. When there is no enough glucose to sustain the growth of bacteria but sufficient lactose is present in the medium, beta-galactosidase synthesis get turned on.

Bacterial sources (4)

- *Lactobacillus bulgaricus*
- *Lactobacillus delbruekii*
- *Lactobacillus casei*
- *Escherichia coli*
- *Bacillus subtilis*
- *Bacillus megatherium*

Fungal sources

- *Alternaria alternate*
- *Aspergillus Niger*
- *Rhizobium sp.*

Yeast sources

- *Candida sp.*
- *Kluveromyces sp.*

Plant sources

- *Apricot*
- *Coffee berries*
- *Peach*
- *Tips of wild roses*

1.2 *Lactose intolerance and Beta-galactosidase*

As lactose intolerant people do not have ability to synthesize galactosidase enzyme, the treatment of milk and it's derivatives with galactosidase is required. Therefore, products free of lactose or low lactose content can be consumed without any problems by lactose

intolerant people (5). Yogurt is usually better tolerated than fresh milk products by maldigesters. This is because the galactosidase in the live yogurt bacteria can aid lactose digestion in the colon. Therefore, Lactic Acid Bacteria (LAB) which constitute a diverse group of lactococi, streptococci and lactobacilli have become a focus of studies (6).Beta-gal mediated trans galactosylation reaction gives di-tri or higher galacto - oligosaccharides (GOS). GSO have a positive impact on the growth and establishment of Bifidobacterium in human intestine and suppress potentially harmful bacteria like Clostridia and Bacteroid species in the gut. Health benefits of GSO include anti-carcinogenic effects, reduction in serum cholesterol, improved liver functioning, and improved intestinal health. Therefore, the public demand for Beta-galactosidase production is significantly increasing together with the development of effective and inexpensive GOS production (7).

Amongst the beta-gal enzymes thus far studied, the Escherichia coli enzyme has been well explored essentially due to its use as a molecular genetics tool, following the discovery of the lactose operon (8). But its industrial use is limited due to the fact that it is not considered safe for food applications. Therefore, selection of microorganisms which are safe for human use and are capable of producing high levels of Beta-gal becomes an urgent and attractive task.

In this regard, Lactic Acid Bacteria and Bifidobacterium, which are generally recognized as safe (GRAS) organisms, have been regarded as good sources of Beta-galactosidase, especially for functional food applications (9).

In addition, microorganisms used to produce the beta-gal have varying nutritional requirements and thus they produce enzymes other than beta-gal such as proteolytic and lipolytic enzymes which can produce inferior organoleptic properties or

other quality defects in milk and milk products (10). Consequently there's a need for new microbial sources that are capable of producing economic quantities of Beta-gal with the ability to function efficiently under various conditions.

MRS agar is the preferred medium for screening of this enzyme producers. X-Gal and IPTG induced blue color colony formation along with the microscopic, biochemical identification procedures are the basic criteria underlying its isolation and identification (11). Since it is an intracellular enzyme, studies on efficiency of different cell disruption methods suggests that lysozyme treatment is the promising one followed by a glass bead treatment (12). Characterization studies concludes that the isolated *Lactobacilli* should survive both the acidic (pH 2.5) and alkaline (pH 8.5) conditions (13). Beta-gal activity is usually determined by enzyme assay procedure, using ONPG (Ortho Nitro Phenyl beta-D- Galactoside) as substrate in the place of lactose. The advantage of using ONPG as the substrate instead of lactose is that, the enzyme cleaves ONPG in to galactose and a chromogenic substance (ONP) having an yellow color, which makes the assay easily observable one. As the enzymatic degradation continues, more and more ONP get released and the solution gets more yellowish. Optical density at 420 is the measure of the amount of ONP present in the sample and in turn the beta-gal activity and this is the principle of this enzyme assay (14).

The probiotic character study generally includes studying growth at different temperature, resistance to low pH, antimicrobial activity, resistance to antibiotics, bile salt resistance etc.

2. Conclusion

Even though both curd and raw milk are sources of industrially important as well probiotic beta-gal producers, any detailed comparison will prefer curd for the first position due the rich amount of probiotic bacteria having sufficient amount of highly active beta-gal production.

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Physiochemical evaluation, phytochemical screening and Pharmacognostic profile of leaves of *Artocarpus hirsutus* Lam. in different solvent extracts

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Abstract

A.hirsutus (wild jack) endoge-neous to Kerala has wide medicinal values which is well documented in the third volume of Hortus Malabaricus. The present work is an attempt to standardize the leaves by pharmacognostic studies and phytochemical analysis. The preliminary phytochemical analysis of the plant extracts was carried out to identify the primary and secondary metabolites present in the various alcoholic and aqueous extracts using standard protocol. Pharmacognostic study includes macroscopic, microscopic studies, physicochemical parameters and florescence analysis. The extraction ability of different solvents for recovering extractable components followed the order: methanol > chloroform petroleum ether > ethyl acetate >distilled water. Phyto-screening of leaves for secondary metabolites revealed the presence of quinone , cardiac glycoside , terpenoids ,flavanoids , phenol, coumarin , alkaloid , steroids and phlobatannin. The macroscopic and microscopic evaluation revealed characters that are of diagnostic value and useful in authentication of the plant. The values of total ash , water soluble ash and acid insoluble ash were 7, 2.33 and 2 % respectively.

Key words: *Artocarpus hirsutus*, standardization, phytochemicals, physicochemical

1. Introduction

As the crude drugs form the basis for the manufacture of wide range of medicinal preparations needed by people, the development of pharmacognostical research has become indispensable for procuring therapeutically potent medicine prepared from genuine drug material. The pharmacognosists have a serious responsibility, to take the initiative not only in correctly locating the plant mentioned in old treatises and pharmacopeias but also making them available to scientists in other disciplines to test the use for which they are acclaimed (Sawant et al,2010).

The value of medicinal plants to the mankind is very well proven. India harbors about 15 percent (3000 – 3500) medicinal plants, out of 20,000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country. It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines (Shanley and Luz, 2003). Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations. Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. These compounds are involved as well in allelopathy, dealing with the interactions between two plants, which process can change depending upon variations in the phytochemicals produced under particular environmental conditions (Zobel *et al*., 1999). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Prusti, 2008).

The interest in nature as a source of potential chemotherapeutic agents continues. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. Higher plants contribute no less than 25% on the total (Cragg and Newman, 2005). In the last 40 years, many potent drugs have been derived from flowering plants.

Plants have been utilized as medicine for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations. The special plants to be used and the methods of application for particular ailments were

passed down through oral tradition. Eventually information regarding medicinal plants was recorded in herbal pharmacopoeias.

Artocarpus hirsutus belongs to the family Moraceae and this family comprises of 50 different species. The name Artocarpus is derived from the Greek words artos (bread) and carpos(fruit) (Bailey,1942). *A.hirsutus* are evergreen and deciduous trees growing in the southern regions of India. It is known by a variety of name s such as Aani, Aini, Aini -maram, Anjili and Anhili found in Karnataka, Kerala and Tamil Nadu. The important source of this plant is edible fruit, leaves, bark and also economically good in timber. *A. hirsutus* is distinctively well known as sources of traditional medicine, food and industry. Also it forms one of the major keystone species of Western Ghats (Nayar 1996,Issac ,2005). Dry leaves are useful in treating bubose and hydrocele. Burn the leaves and the ash is taken internally to treat abdominal problems by Paniya, Kurichia,Adiyan, Kattunaika tribes of Wayanad district (Shyma and Devi Prasad, 2012)

There are no reports on the pharmacognostical studies of leaves . Hence, the present work is an attempt in this direction to standardize the leaves by pharmacognostic studies and phytochemical analysis, as these studies stand as evident proof for confirming its identity, authenticity and purity of the plant.

2. Materials and methodology

2.1 Collection of plant materials.

The fresh leaves of *Artocarpus hirsutus Lam*.were collected in the month of December 2016 from the Kurumal, *Thrissur* Kerala, India. Taxonomic identification made with Flora of the Presidency of Madras by JS Gamble. The plant name checked with www.theplantlist.org.

2.2 Preparation of extracts

Leaves of the plant were shade dried for several days. The dried plant material was ground to a course powder and 50 gm of the powdered plant material was soaked in solvents of increasing polarity starting petroleum ether, chloroform, ethyl acetate, methanol and distilled water (1:5)

for 72 hours .The solvent was then removed by evaporation. Each residue was weighed and the yield percentage was determined. Percent of yield was calculated as follows:

Extract yield %= $(W_1/W_2)\times 100$

Where, W_1 is net weight of powder in grams after extraction and W_2 is total weight of wood powder in grams taken for extraction.

The dried extract was stored in refrigerator for further studies.

2.3 Macroscopic study

The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens.

2.4 Microscopic study

Free hand transverse sections of leaf and petiole were taken and stained with safranin and observed for their peculiar characters.

2.5 Determination of stomatal index

As it is difficult to obtain satisfactory epidermal peelings of the leaves of *A.hirsutus*, a simple "fevicol impression method" developed by Bai and Jose(1981) in cassava was successfully adopted for the study of stomatal characters.

Stomatal index is the percentage of the number of the stomata to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following formula.

S. I = S/ E+S X100

Where, S.I= Stomatal index

S= Number of stomata per unit area

E= Number of epidermal cells in the same unit area

2.6 Physicochemical parameters (Kokate, 2003)

Ash values (Total ash, acid insoluble ash, water soluble ash) were determined. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material.

2.7 Fluorescence analysis

A small quantity of dry plant powder is placed on grease free clean petriplate and 1 or 2 drops of freshly prepared reagent solution is added, mixed by gentle tilting the petriplate and wait for few minutes. Then the petriplate is placed inside the UV chamber and observe the colour in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations is recorded . Generally the colour change is noted in reagents like Powder + Distilled water, Powder + $CuSO_4$, Powder + 1 M NaOH, Powder + Picric acid, Powder + acetic acid, Powder + dil. HCl, Powder + 5% Iodine, Powder + 5% $FeCl_3$, Powder + HNO_3 + 25% NH_3, Powder + Methanol, Powder + 50% HNO_3, Powder + Conc.H_2SO_4, Powder +Conc.HNO_3, Powder + Liquid ammonia etc. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluorescent in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs.

2.8 Phytochemical Screening

The preliminary phytochemical analysis of the plant extracts was carried out to identify the primary and secondary metabolites present in the various alcoholic and aqueous extracts of leaf of *A. hirsutus* using standard protocol given by Harborne, 1993.

3. Result

3.1Macroscopic characteristics

A large tree with branchlets horizontally drooping and hairy (Fig 1). Leaves simple, clustered at twigs end., alternate, broadly egg-shaped or elliptic. stipules to 4 cm long, lateral, ; petiole 10-30 mm long 12-25x 7-15 cm, base rounded , apex bluntly acute, margin entire and wavy, leathery, with sparsely scattered hairs above, densely hairy below (Table 1). Male and female flowers very minute, numerous, greenish

yellow, separately seen in different heads of the same plant in the leaf axils. Mature fruits globose to avoid, sorosis, covered by numerous long spine-like projections, ripening orange-yellow.

3.2 Macroscopic characteristics

3.2.1Transverse section of petiole

Outline of the petiole is almost round. Outer epidermis with cuticle followed by heterogeneous cortex with chlorenchyma and paremchyma. Within the ground tissue secondary vascular tissues forms a continuous ring. It is conjoint, collateral and open . Pith consists of parenchyma cells. (Fig.2 a &b)

3.2.2 Transverse section of lamina

Thin walled rectangular cells forms upper and lower epidermis and it is cuticularised. Anisocytic stomata present on both surfaces. Upper surface contains trichomes. Mesophyll tissue is divided into compactly arranged, columnar palisade and loosely arranged spongy tissues. Well-developed vascular bundles are also seen (Fig 2 c&d). Stomatal index of upper leaf surface and lower surface are 20.06 and 17.06 respectively.

3.3 Physicochemical Parameters

Leaves were subjected to ash test and results presented in Table 2.

3.4 Fluorescence analysis

Result of fluorescence analysis are presented in Table 3.The characteristic appearence of powder upon treated with various chemicals and their difference in daylight and UV lights are important diagnostic characters for the proper idendification of crude drug in its died and powdered form.

3.5 Yield of extract

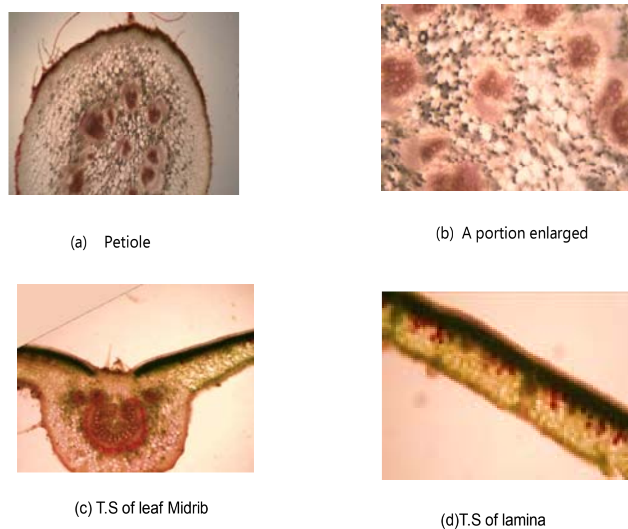
Comparatively, methanol extract exhibited higher extraction yield (1.78g) . The extraction ability of different solvents for recovering extractable components followed the order: methanol > chloroform petroleum ether > ethyl acetate >distilled water (Table 4).

3.6 Preliminary Phytochemical Screening

Phytochemical screening results (Table 5) of the leaf extracts in petroleum ether showed the presence of carbohydrate, sugar, protein, flavanoids, and coumarin. Chloroform extract contained ketose,

protein ,quinine , flavanoids and coumarin. Metabolites present in ethyl acetate are protein, quinine and tannin. Sugar, carbohydrate, protein, cardiac glycoside, steroids, , flavanoids, alkaloid, tannin and phlobatannin are present in methanol extract. Metabolites like sugar, protein, quinone, cardiac glycoside, flavanoid , tannin, terpenoid , coumarin and phenol are present in aqueous extract.

Figure 1: Habit of *Artocarpus hirsutus*



(a) Petiole

(b) A portion enlarged

(c) T.S of leaf Midrib

(d)T.S of lamina

Fig 2 Microscopic characters of leaves of *Artocarpus hirsutus*

Table 1: Morphology of the leaf of *Artocarpus hirsutus*

Morphological parameters	Observation
Condition	Fresh
Type	Simple
Phyllotaxy	Alternate
Length of lamina	23-25 cm
Width of lamina	13-15 cm
Venation	Reticulate
Leaf shape	Obovate
Surface of the leaf	Glabrous
Tip of lamina	Obtuse
Surface of leaf sheath	Glabrous

Table 2. Physico chemical parameters of leaves of *Artocarpus hirsutus*

Parameters	Values
Total ash	7%
Water soluble Ash	2.33%
Acid soluble Ash	2%

Table 3. Fluorescence analysis of leaves of *Artocarpus hirsutus*

Treatment	Fluorescent light	UV short	UV long
Powder As Such	Green	Pale green	Brownish green
Powder + Water	Dark green	Light green	Black
Powder + $CuSO_4$	Dark green	Blackish green	Black
Powder + 1m NaOH	Black	Brownish black	Black
Powder + 1%Picric Acid	Pale green	Dark green	Brown
Powder + Acetic Acid	Yellowish green	Dark green	Brown
Powder + HCl	Brownish black	Dark green	Black
Powder + 5%Iodine	Brownish green	Dark green	Black
Powder + 5%$FeCl_3$	Black green	Blackish green	Black
Powder + HNO_3+ 25%NH_3	Reddish orange	Brownish green	Black
Powder + Methanol	Green	Dark green	Brownish black
Powder + 50%HNO_3	Reddish orange	Brown	Black
Powder + H_2SO_4	Black	Black	Black
Powder + Conc.HNO_3	Reddish orange	Yellowish black	Black
Powder + Liquid NH_3	Brown	Yellowish black	Brown

Table 4. Percentage yield of *Artocarpus hirsutus*

Petroleum ether	Chloroform	Ethyl acetate	Methanol	Distilled water
0.817%	1.12%	0.70%	1.78%	0.67%

4. DISCUSSION

In the present investigation, the detailed macroscopic and microscopic characters were studied which will be helpful for the correct botanical identification. Phytochemical study is helpful to isolate the pharmacologically active principles present in the drug.

Mixture of such chemicals shows a spectrum of biological effects and pharmacological properties. Tannins bind to proline rich proteins and interfere with the protein synthesis. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Phenolic is one of the major groups of phytochemical that can be found ubiquitously in certain plants. Phenolic compounds are potent antioxidants and free radical scavenger which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers. Studies have shown that phenolic compounds such as catechin and quercetin were very efficient in stabilising phospholipid bilayers against peroxidation induced by reactive oxygen species (ROS).

It is concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. If the local ethno-medical preparations and prescriptions is scientifically evaluated and disseminated properly the botanical preparation of

traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery. Towards authentication and quality assurance of medicinal plants, pharmacognostic, physicochemical and preliminary phytochemical studies of the *A. hirsutus* were carried out. The macroscopic and microscopic evaluation revealed characters that are of diagnostic value and useful in authentication of the plant. Physicochemical analyses reveals values for Total ash , water soluble ash and acid insoluble ash are within the World Health Organisation (WHO) standards for crude drug from medicinal plants.

Phyto-screening of leaves for secondary metabolites revealed the presence of quinone, cardiac glycoside, terpenoids ,flavanoids, phenol,coumarin , alkaloid , steroids and phlobatannin. Information obtained from these studies can be used as markers in the identification and standardization of this plant as a herbal remedy and also towards monograph development of the plant.

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Table 5. Qualitative analysis of the phytochemicals of leaves of *Artocarpus hirsutus*

Primary/Secondary metabolites	Name of test	Petroleum ether	chloro-form	Ethyl acetate	Methanol	Distilled water
Carbohydrates	Molisch's Test	+	–	–	+	–
Aldehyde	Fehling Test	–	–	–	–	–
Starch	Iodine Test	–	–	–	–	–
Sugar	Benedict's Test	+	–	–	+	+
Ketose	Seliwanoff's test	–	+	–	–	–
Proteins	Lowry's Method	+	+	+	+	+
Aminoacid	Ninhydrin test	–	–	–	–	–
Fats	Filter paper Test	–	–	–	–	–
Quinone	$H2SO_4$ Test	–	+	+	–	+
Cardiac glycoside	Kellar –Killani test	–	–	–	+	+
Steroids	Salkowski test	–	–	–	+	–
Flavonoids	Fluorescent Test	+	+	–	+	+
Phenols	Folin Test	–	–	–	–	+
Saponins	Foam test	–	–	–	–	–
Alkaloids	Wagmens test	–	–	–	+	–
Tannin	Iron salt test	–	–	+	+	+
Phlobatanin	Conc. Hcl test	–	–	–	+	–
Terpanoids	Salkowski test	–	–	–	–	+
Acid	NaHCo3 test	–	–	–	–	–
Coumarin	Fecl3 test	+	+	–	–	+

+ indicate the presence of constituents and — indicate the absence of constituents

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Phytochemical analysis and antimicrobial effect of *Azadirachta indica, Psidium guajava and Spinacia olerocea* against *Propionibacterium acne*

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Abstract

Acne vulgaris is the most common disorder of human skin that affects upto 80% of individuals in their lives.The hypersecretion of hormone androgen stimulates higher sebum secretion in sebaceous gland. The daily use of many cosmetics makes skin dull and the chemical contents have their own negative effects. This study is attempt to findout the disease control potency of phytochemicals against acne causing bacteria ***Propinibacterium acne.*** Hot water extract of ***Psidium guajava, Azadiracha indica*** and ***Spinacia oleracea*** are used for the study. Different concentrations of extracts such as 1500 μg/ml, 1000μg/ml, 500μg/ml and 100μg/ml are used for the study. Phytochemical analysis showed the presence of alkaloids, steroids, flavonoids, tannin and saponin. Agar Well diffusion method is used for the detection of antimicrobial activity. Among the three plat extract ***Azadiracha indica*** showed excellent activity. ***Azadiracha indica*** can be used in the treatment of infectious disease caused by ***P.acne.***

Key words*: Phytochemicals , Agar Well diffusion*

1. Introduction

Acne is a skin disorder for which dermatologists are still struggling since years to treat successfully.It mainly affects adolescents though it may present at any age. Acne by definition is multifactorial chronic inflammatory disease of pilosebaceous units.It affects the skin of the face,neck and upper trunk.These particular sebaceous follicles have capacious follicular channels and voluminous, sebaceous glands.Acne develops when these specialized follicles undergo alteration that results in formation of noninflammatory

lesion(concedons) and inflammatory lesions(papulese,postules and nodules) black heads and wrinkles are also seen in people suffering from acne vulgaris. (GM White,1999)

Staphylococcus epidermis and *Propionibacterium acne* are considered as the major skin bacteria that cause the formation of acne.P.acne is one among the gram positive microaerophillic anaerobic bacilli plays and it is implicated in development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids which chemotactically attracts neutrophils.(G.SKumar*et.al* .,2007).

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform biological functions and to defend against attack from predators such as insects,fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects are long term health when consumed by

humans and can be used to treat human diseases. The ingredients in tropical acne treatments particularly herbs and naturally derived compounds have received considerable interest as they have fewer adverse effects than synthetic agents.In present study three essential plant extracts of *Azadirachta indica, Psidium guajava, Spinacia olerocea* were prepared which have been traditionally used as antimicrobial and anti inflammatory agents were examined for antimicrobial activities against *P.acne.*

Psidium guajava are very efficient in treatment of acne and black spots.Guava leaves are effective in eliminating acne and black spots from the skin.They contain an antiseptic that can kill acne causing bacteria.For the treatment of blackheads blend guava leaves with water and use it as a scrub on face to remove blackheads.Guava leaves contain antioxidants which destroy the free radicals that damage skin,thus protecting skin from aging as well as improving skin tone and texture.A decoction of mature guava leaves can be applied on the skin for tightening it.Guava leaves are an instant cure for getting rid of itchiness as they contain allergy blocking compounds.

The available products that are currently in the market gaining it's significance in providing relief for acne or pimple problems are unable to prevent the

reoccurance. The current research focuses on antibacterial potency of some medicinal plants against acne causing organism *Propionibacterium acne.*

2. Materials and Method

2.2 The collection and processing of plant extract

The three plants used in this study were collected from local surrounding of Thrissur District,Kerala . The leaves were thoroughly washed in water and air dried in room temperature for one week. They were grind into powder and subjected to extraction with hot water. The crude powdered plant material of 10g was washed and extracted with hot water in a large beaker.The extract was prepared by continuous boiling water for 810 hrs. The extracts were concentrated and air dried. The samples were then stored in airtight bottles at 4°c.

2.1 Isolation and Identification of Propionibacterium acne.

The skin was first wiped with ethanol and the material was extracted from skin lesions and took up by sterile swab sticks and inserted into Tryptone yeast extract glucose agar(TYGA). The *Propionibacterium acne* culture was maintained in tryptone yeast extract glucose agar (TYGA) plates and TYG broth. The mother inoculum was maintained anaerobically using candle jar about 2 days.

The isolate was identified as *Propionibacterium acne* by using various biochemical test like Catalase test, Indole test, Nitrate test and Carbohydrate fermentation tests,

2.3 Phytochemical analysis

Test for alkaloids, flavonoids, steroids, phenols, tannins, glycosides, terpenoids, saponins, anthraquinones and cardiac glycosides (kellerkillani test) were performed for the detection of secondary metabolites.

2.4 Anti microbial Assay

The plant extracts of different plants were tested against the pimple causing bacteria *Propionibacterium acne* using well diffusion method. About 5 wells in each plates of 6mm diameter were punched in TYG Agar surface for placing the plant extract in a concentration of 1500 mg/ml, 1000mg/ml, 500mg/ml, 100mg/ml. Aqueous extracts of 3 plants were mixed with 2%DMSO and added in separate places in consent. Clindamycine is used as control. The plates were kept for

incubation for about 24 to 48 hours. After 48 hours of incubation the zone of inhibition was clearly visible and the diameter of the zone was measured and tabulated.

3.Result

Colonies isolated in modified TYGA medium, the first colony isolation identified as ***Propionibacterium acnes.*** It grow on BHA (Brain Heart infusion Agar) medium. Colony showed a cream colored raised spread growth. Gram staining and motility test showed that it is motile gram positive rod shaped bacterium. It do not produce endospore.Indole test, nitrate test, catalase test and sugar fermentation test are carried out. The organism showed positive result to indole, nitrate and catalase test. It can also ferment glucose.

Phytochemical analyses of aqueous extracts of these plants were carried out by using standard procedure. The tests were based on qualitative analysis. Phytochemical screening revealed the presence of primary and sacondary metabolites. The primary metabolites like carbohydrate, reducing sugars and lipids are found in each plants. The secondary metabolites alkaloids, steroids, flavonoids, tannin and saponin are found in *Azadirachta indica* leaf, steroids, flavinoids, tannins and cardiac glycoside are found in *Psidium guajava leaf* and phenol, flavinoids, tannin, glycoside and saponin are found in *Spinacia oleracea* seed.

Antibacterial testing of aqueous extracts of the three plants against *P. acnes* was done by well diffusion method. After incubation, a zone of inhibition observed in the plates and indicated the antibacterial activity of these plants against *P. acnes*(Swanson ,2003)

Based on the aqueous plant extract and their concentrations they showed different zones of inhibition. The different concentrations are 1500 µg/ml, 1000µg/ml, 500µg/ml and 100µg/ml. The aqueous extracts of *Azadirachta indica* showed a zone of inhibition 20 mm, 18mm, 17mm and 15mm. The aqueous extracts of *Psidium_guajava* showed a zone of inhibition 20 mm, 15mm, 14mm and 11mm. The aqueous extracts *of Spinacia oleracea* showed a zone of inhibition 16,14,13 and 12mm.

4.Discussion

Azadiracha indica showed excellent antibacterial activity against the bacteria

and showed the presence of alkaloides, steroids, flavanoids and tanins. Flavanoids and tanins present in the extract contributed the major antibacterial activity. Some of these secondary metabolites are also present in the *Psidium guajava* and *Spinacia oleracea* also have anti bacterial property against *Propionibacterium.*

Alkaloids, terpenoids, phenolics and pigments can have therapeutic actions in humans and which can be refined to produce drugs. These phytochemicals present in plant play a vital role in contributing them the antiacne property.(Nand *et al*., 2002)

5.Conclusion

The systemic research for useful bioactive agent from the plants is now consider to be a rational approach in nutraceuticals and drug research.The results of phytochemical analysis comprehensively validate the presence of therapeuticaly important and valuable secondary metabolites. Acne induced by

Propionibacterium represents a fastidious cutaneous problem .To get rid of this problem, large amount of money is spent every year on medicins and other agents world wide but, attempt to completely remove this problem are still a far of target. Our present studies indicates that the medicinal plant extract inhibits antibacterial activity. Thus they can be used in the treatment of infectious disease caused by *P.acne*.(Mengesha *et al* ,2002)

The present investigation demonstrated that extracts of premedicinal plants *Psidium guajava, Azadiracha indica, Spinacia oleracea* showed excellent antibacterial activity against the bacteria. so the phytochemicals present in plants play a vital role in contributing them the antibacterial action. So the combined effects of these plants can provide effective medicinal value and can be used as a drug for skin disease causig organism *P.acne.* To check effectiveness of botanicals, detailed biomolecular, characterisation, formulation of dosages, clinical studies, safety and efficiency a lot of more has yet to be explore. It is concluded that these plants could be a potential source of active antibacterial agents

Table-1-The result of preliminary screening for secondary metabolites of hot water extract

Sl No	Hot water extract	Secondary metabolites										
		A	F	St	P	T	G	S	Aq	Cg	Tr	Co
1	*Spinacia olerocea*	+	+	+	-	+	+	+	+	-	-	+
2	*Psidium guajava*	-	+	+	-	+	-	+	+	-	-	-
3	*Azadirachta indica*	+	+	-	-	+	-	+	-	-	-	-

+ Presence of secondary metabolites, - Absence of secondary metabolites.A-Alkaloids,F-Flavanoids,St-Steroid,P-Phenols,T-Tannin,G-Glycoside,S-Saponin,Aq-Anthraqunone,Cg-Cardiacglycoside,Tr-Terpenoids,Co-Coumarin.

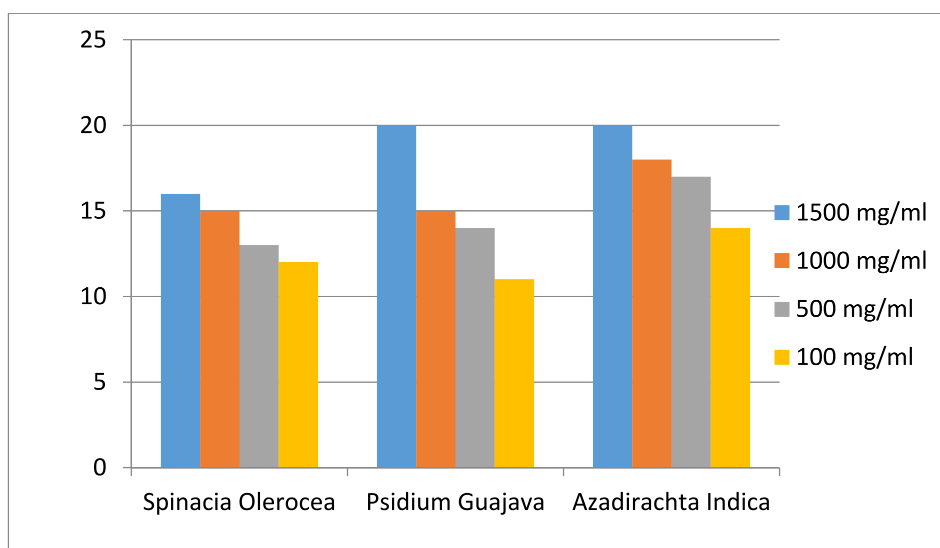

Figure 1: Graph showing the effects of medicinal plant extracts on *P.acne*

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Comparative study on phytochemical, physiochemical and antioxidant properties of *Sorghum bicolor* and *Triticum aestivum*

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Abstract

Increased consumption of whole grains has been associated with reduced risk of major chronic diseases including cardiovascular disease, type II diabetes, and some cancers. The unique phytochemicals in whole grains are responsible for the health benefits of whole grain consumption. ***Triticum aestivum*** (Wheat) is a major crop and an important component of the human diet, particularly in developing countries. ***Sorghum bicolor*** (Sorghum) is an important cereal widely cultivated in tropical and subtropical regions. The objectives of this study were to compare the phytochemical profiles, physiochemical properties and antioxidant activities of sorghum and wheat. The phytochemical studies revealed the presence of various phytochemical such as carbohydrates, protein, tannins, flavonoids, terpenoids, steroids and alkaloids etc. The results showed the gluten content is low in sorghum (7.855%) when compared to that of wheat (14.935%). Total ash content (17.92% and 15.41% for sorghum and wheat, respectively) and moisture content (15.73% for sorghum and 12.18% for wheat) were also determined. Sorghum had the high total antioxidant activity with EC_{50} value 55.90 µg/mL and that of wheat is 47.56 µg/mL. The methanolic extracts reduced the DPPH radical in a dose dependent manner with 50% reduction in 21.51 and 27.69 µg/mL for sorghum and wheat respectively. Total reducing capacity is with response to the increase in drug concentration in both extracts with EC_{50} value of 27.245 and 49.81 µg/mL for sorghum and wheat, respectively. The study showed that the sorghum and wheat-based diets can serve as functional foods, as evident from their antioxidant capabilities in addition to their gross energy content.

Keywords: *Phytochemicals; Gluten content; antioxidants; wheat; sorghum*

1. Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). Medicinal plants are valuable natural resources and regarded as potential safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in modern medicine (Hassawi and Kharma 2006, Bhat *et al*., 2009). The phytochemical analysis of the plant is very important commercially and has great interest in pharmaceutical companies for the production of many new drugs for curing various diseases. Phytochemicals such as alkaloids terpenoids and flavonoids exhibit various important pharmacological activities such as anti-inflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antimicrobial activities (Hagerman *et al*., 1998). Dietary phytochemicals are commonly found in plant-based foods such as fruits, vegetables, grains and tea (Probst *et al*., 2015). Whole grains in our food have a role with reduced risk of major chronic diseases including cardiovascular disease, diabetes, and some cancers. Whole grains are rich in nutrients and phytochemicals with known health benefits. Whole grains are enriched in antioxidants including trace minerals and phenolic compounds and these compounds have been responsible for disease prevention. It also contains phytate, phyto-oestrogens such as lignan, plant stanols and sterols, and vitamins and minerals. The unique phytochemicals in whole grains are believed to be responsible for the health benefits of whole grain consumption (Slavin, 2004).

Sorghum bicolor (family Poaceae) is the 5th most important cereal globally in terms of acreage and production. Most of the phytochemicals in sorghum are excellent antioxidants. Sorghum has great drought-tolerence. Sorghum is gluten free and thus can be consumed by people with celiac disease. *Triticum aestivum* (Wheat) is a major crop and an important

component of the human diet, particularly in developing countries. Epidemiological studies have associated the consumption of whole grain and whole-grain products with reduced incidence of chronic degenerative diseases (De Moura, 2008). Accordingly, dietary-plant-based drug discovery still remains an important area, remains unexplored, where a systematic search may definitely provide important leads for the pharmacological benefits.

2. Materials and methods

2.1 Plant material and extraction: The fresh and healthy grains of Sorghum *bicolor* and *Triticum aestivum* were collected from the market Kunnamkulam, Kerala. The fresh grains were washed, dried in shades. The dried samples were then crushed into powder. The crude powdered plant material of 20 g was extracted with 200 ml methanol and stirred it using magnetic stirrer overnight. After extraction the solvent was removed to yield a viscous residue of extract. The extract was filtered through Whatmann No.1 filter paper. The extract were then concentrated at 40-45^0 C in hot air oven and dried.

2.2 Chemicals

2, 2-diphenyl-1-picryl hydrazyl (DPPH) is procured from Sigma Aldrich. Nitric acid, potassium di hydrogen phosphate, potassium ferrocyanide, sodium hydroxide, sodium phosphate and sulphuric acid were purchased from MERK specialities Pvt Ltd., Mumbai. All other chemicals and reagents used were of analytical reagent grade and purchased from reputed Indian companies.

2.3 Methods

2.3.1 Phytochemical screening:

Phytochemical screening of various extracts of U. narum leaves was carried out according to standard procedures (Smitha *et al.*, 2014)

2.3.2 Physiochemical Properties

A.Determination of Percentage Gluten in flour:

About 25 g of each composite flour sample was weighed in to a petridish of known weight and thoroughly mixed with 1 ml of water to form dough .The dough is kneaded under running water to remove starch and later put in to petridish and

weighed .It was then dried in an oven and weighed after drying.

*% Dry weight = Dry gluten weight / Dry flour weight *100*

A. Ash Determination of extracts:

Ash determination was by method of ADAC (1990). Two grams of plant powder was weighed crucible .The crucible and content were ignited in a preheated furnace to 600 degree for 2 hours. The crucible was loaded in a desiccator, was weighed and percent ash content was calculated

*Ash content =Weight of ash /Weight of sample * 100*

A. Moisture Content Determination:

Moisture was determined by the method of AOAC 1990. Two grams of plant powder was weighed in to pre-dried and weighed crucible and placed in thermostatically regulated hot air oven at 169 degree for 9 hour. The crucible was removed dried in a desiccator and weighed. And the process was repeated until a constant weight was obtained; moisture content was regulated and expressed as a percentage of initial weight of the flour.

Moisture % = (Initial mass of sample – Mass after drying) /weight of sample used

2.3.3 Antioxidant activities

A. DPPH radical scavenging activity:

The scavenging activity of the extract on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was analyzed by the method of (Aquino *et al.*, 2001). DPPH, in its radical form has an absorption peak at 515 nm, which disappears on reduction by an antioxidant compound, evident from its transition from purple to yellow colour. DPPH, in solution was incubated in the presence of extract at concentrations for 20 minutes at room temperature in the dark and absorbance was then read. Assay was carried out in triplicate. The IC_{50} value was determined.

B. Total antioxidant capacity assay:

The total antioxidant capacity was measured according to spectrophotometric method of Preito *et al;* (1999). Various concentrations of crude extract dissolved in distilled water

were combined with 1 ml of reagent solution containing 0.6 M sulphuric acid, 2.8 mM sodium phosphate and 4mM Ammonium molybdate. The tubes were capped and incubated at 90^0 C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against blank. The activity of the drug was compared with that of control.

C. Total reducing power activity :

The reducing power of sequential extracts was determined by the method of Yen and Duh (1994). Different concentrations of extracts were mixed with 2.5 ml of phosphate buffer (200 mM, p^H 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated for 20 minutes at 50°C. After incubation, 2.5 ml of 10 % Trichloroacetic acid were added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm. The activity of the drug was compared with that of control.

2.4 Statistical analysis

All *in vitro* assays were performed in triplicate and data represented are mean ± SD.

Table 1: Phytochemical screening of *Sorghum bicolor* and *Triticum aestivum*

Phytochemicals	Sorghum	Wheat
Carbohydrates	+	+
Protein	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+
Steroids	+	+
Alkaloids	+	+
Saponins	+	+

3.Results

3.1 Phytochemical analysis of Sorghum bicolor and Triticum aestivum

Phytochemical screening of *Sorghum bicolor* and *Triticum aestivum* methanolic extracts revealed the presence of protein, phenolic compounds, alkaloids, terpenoids, saponins and tannins (Table 1).

3.2 Physiochemical properties of Sorghum bicolor and Triticum aestivum

A. Determination of Percentage Gluten in Sorghum bicolor and Triticum aestivum Flour

The gluten content of *Sorghum bicolor* and Triticum aestivum flour were determined and the results were shown in Figure 1A.The results showed that sorghum has low gluten content compared to wheat.

B. Ash Determination of Sorghum bicolor and Triticum aestivum Flour

The ash value of Sorghum bicolor is 17.92% and that of *Triticum aestivum* is 15.41% (Figure1B).

C. Moisture Content of Sorghum bicolor and Triticum aestivum Flour

The moisture contents of *Sorghum bicolor* and *Triticum aestivum* were 15.73 and 12.18% respectively. Results are depicted in Figure 1C.

3.3 Antioxidant activities of Sorghum bicolor and Triticum aestivum

A.DPPH radical scavenging activity of Sorghum bicolor and Triticum aestivum

The methanolic extracts of *Sorghum bicolor* and *Triticum aestivum* showed potent DPPH radical inhibition. The maximum activity was obtained in tested concentration of 30 µg/mL showed 62.5 and 58.33% inhibition for sorghum and wheat respectively. IC_{50} value of methanolic extract was reported as 21.51 and 27.69 µg/mL for sorghum and wheat respectively. This indicates significant DPPH inhibition of *Sorghum bicolor* and *Triticum aestivum* extracts (Figure 2A).

B. Total Antioxidant activity of Sorghum bicolor and Triticum aestivum

The results show the increase in

antioxidant activity with response to the increase in drug concentration. EC_{50} value of methanolic extract of sorghum was reported as 55.90 µg/mL and that of wheat is 47.56 μg/mL. (Figure 2B).

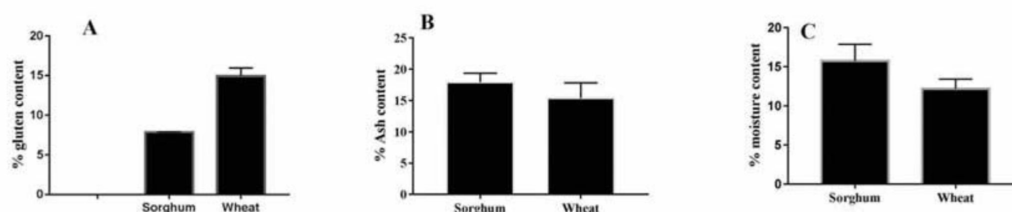

Figure 1 .The A) % Gluten content B) % ash content and C)% moisture content of *Sorghum bicolor* and *Triticum aestivum*

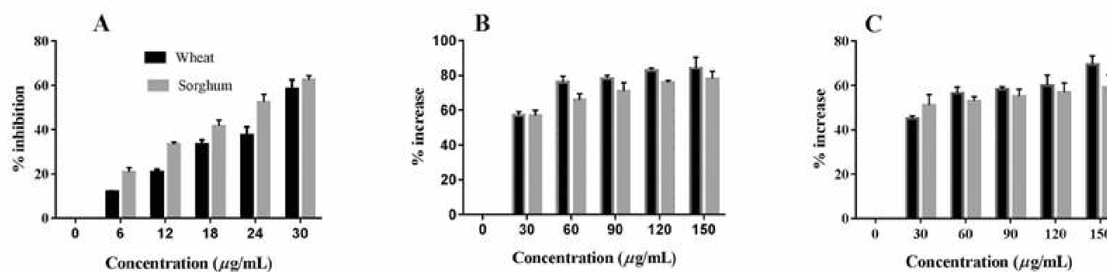

Figure.2. A)DPPH radical scavenging activity , B) Total antioxidant activity C) Total reducing capacity of methanolic extracts of *Sorghum bicolor* and *Triticum aestivum.*

C. *Total Reducing Capacity of Sorghum bicolor and Triticum aestivum*

The methanolic extracts of *Sorghum bicolor* and *Triticum aestivum* was showed total reducing capacity. The observation was show the increase in total

reducing capacity with response to the increase in drug concentration. EC_{50} value of the extracts were reported as 27.245 µg/mL for sorghum and 49.81 µg/mL for wheat respectively (Figure 2C).

4.Conclusion

The present study revealed the antioxidant property of *Sorghum bicolor* and *Triticum aestivum* which can defend against free radicals. It was found that dietary grains *Sorghum bicolor* and *Triticum aestivum* possess numerous phytoconstituents. The free radical scavenging and antioxidant activities of the grain extracts supported the evident of identified phytochemicals present in it. The gluten content is very less in the case of *Sorghum bicolor compared to Triticum aestivum*. So it can use in disease condition such as celiac disease.

This reveals the antioxidant activities of methanolic extracts of *Sorghum bicolor* and *Triticum aestivum* which can be used as a potential dietary substance as well as pharmacological agent.

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State the **Conclusions** in a few sentences at the end of the paper. The main conclusions of the study may be presented in a single paragraph.

The **Acknowledgments** of people, grants, funds, etc should be brief.

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Mini reviews

The format requirements for original research papers apply to reviews too.

Submission

Submission in electronic form of the final version of the manuscript to the email id **abassmc@gmail.com**.

Annals of Basic and Applied Sciences

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The manuscript must be typed in Times New Roman, font size 12, double-spaced and all pages should be numbered starting from the title page. Headings should be Times New Roman, small letter, bold. Font size 12. Sub headings should be Times New Roman, small letter, italics and without bold. Font size 12.

The main sections should be numbered 1, 2 etc., the sub-sections 1.1, 1.2, etc., and further

subsections (if necessary) 1.1.1, 1.1.2, etc.

The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include

• Concise and informative title. (Times New Roman. Text font size 16)

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Structured **Abstracts** are required for all papers and should include objectives, key findings and major conclusions. It should be a single paragraph with not more than 250 words. References should be avoided in abstract.

Following the abstract, about 3 to 6 **key words** should be listed.

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The **Introduction** should provide a background to the study and should clearly state the specific aims of the study. It should be understandable to the audience from a broad range of scientific disciplines. Approximate length is 500-1000 words.

Materials and methods should be complete enough to allow experiments to be reproduced. Methods in general use need not be described in detail. Subheadings should be used. Please include details of ethical approval in this section. Approximate length: 500-1000 words.

Results should be clear and concise with Graphs or Tables, may be inserted along with the matter or may be given as separate with the detailed title and in that case the places where the figures are to be inserted should be mentioned in the manuscript. Each figure and table should be numbered in Arabic numerals and mentioned in the text. Figures must be clearly lettered and suitable for reproduction to fit either one column width (8.5 cm) or two columns width (17 cm). Black and white photographs only are acceptable. The lettering in the figures should be readable. In addition to the inserted version in the word document, the figures can be supplied in electronic format as JPEG or TIFF. Tables should be kept to a minimum and be designed to be as simple as possible. Each table should be on a separate page, numbered consecutively (Table 1, Table 2 etc) and supplied with a heading and a legend. Tables should be prepared in Microsoft Word. The same data should not be presented in both table and graph forms.

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