Profiling of Phenolics, Proteins, Antioxidant Activity and GC-MS analysis of the seeds of *Artocarpus hirsutus.*

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Abstract: **Phenolics, proteins and antioxidant activity profiling of *A. hirsutus* seeds were carried out using different aqueous solvents such as 50mM acetate buffer pH 5.0, 50mM phosphate buffer pH 7.0, 50mM tris buffer pH 8.5, 0.1M sodium hydroxide (NaOH), 0.1M sodium chloride (NaCl), water and organic solvents like ethanol, methanol and acetone. Among the aqueous solvents used, 0.1M sodium hydroxide extract showed maximum amount of proteins, phenolics and antioxidants extraction. Thus different concentrations of NaOH (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5) were chosen for the extraction, of which 0.1M NaOH showed greater extraction efficiency. Among the organic solvent, ethanol showed maximum phytochemical extraction. Thus different concentration of ethanol (20%, 40%, 60%, and 80%) in combination with 0.1M NaOH was used. Among the various solvents and the concentrations chosen, 40% ethanol in 0.1M NaOH showed greater extractability of phenolics, proteins and antioxidants. Thus organic solvent in alkali can be considered as the efficient extraction system. Phytochemical profiling of various seed extracts was done by GC- MS analysis using hexane, ethyl acetate, methanol and water.**

IndexTerms - Artocarpus hirsutus, **organic and aqueous solvents, phenolics, proteins, antioxidants, GC-MS analysis.**

1 INTRODUCTION

Artocarpus hirsutus known as wild jack belong to the family Moraceae (mulberry family) which comprises of about 60 genera and over 1000 species. They are deciduous and evergreen tall tree found in the Southern regions of India. *A. hirsutus* are characterized by the white latex and the fleshy fruit with lots of seeds. Each fruit weighs around 250-500g with golden colored pulp containing 20-25 seeds per fruit. The fruits, roots, buds and leaves of the plant are used in the treatment for malarial fever, liver cirrhosis, hypertension and diabetes **(Jagtap & Bapat 2010, Zhao *et.al.*, 2009**). *A. hirsutus* is rich in flavanoids, saponins, alkaloids and terpenoids **(Vinay *et. al.*, 2014, Sirisha *et. al.*, 2013).** The seed oil is a contains mixture of fatty acids like 9,12-octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) and 10,13- octadecadienoic, methyl ester ($C_{19}H_{34}O_2$) **(Ahmedullah & Nayar 1986 , Prior *et. al.*, 2003)**. Other important species belonging to this genus are *A. heterophyllus, A. altilis, A. hirsutus, A. lakoocha, A. camansi.* Artocarpus contain many bioactive phytochemicals which possess various pharmacological activities **(Jeyam *et. al.*, 2013).** The methanolic fruit extract of *A. hirsutus* revealed potential antioxidant activity **(Akhil, *et. al.*, 2014).** The *A. hirsutus* Lam. bark and root acts as a good antimicrobial agent **(Dibinlal *et. al.*, 2010, Vinay *et.al.*, 2013).** Roots and bark decoctions of *A. hirsutus* were used to cure diarrhea and leaves are used in the treatment for venecosal bubones and chronic hemorrhage. Fruit and bark extract possess antioxidant and anti-ulcer, anti-inflammatory and melanogenesis inhibition properties respectively **(Nayak *et.al.*, 2017).**

Plants are natural antioxidants and are beneficial for human health. The phenolics act as a major contributor for the antioxidant potential of plants **(Carocho & Ferreira 2013).** GC-MS analysis helps in identifying various phytocompounds which possess medicinal properties that can be used in the drug discovery. The present study was designed to explore the total phenolics, protein and antioxidant activity of various extracts of *A. hirsutus* seeds and the estimation of the bioactive components present in them using GC-MS analysis.

2 MATERIALS AND METHODS

2.1 Collection of Plant Material

The wild jack fruit (*A. hirsutus*) was collected from different parts of Mangalore District, Karnataka in the month of April 2017.

2.2 Chemicals

Acetic acid, acetone, ascorbic acid, Bovine Serum Albumin, ethanol, ferric chloride, Folin – Ciocalteau reagent, gallic acid, methanol, sodium hydroxide, sodium chloride, sodium carbonate, sodium dihydrogen phosphate, disodium hydrogen phosphate, tris, potassium ferricyanide, , trichoroacetic acid, , were purchased from SRL Chemicals.

GC/MS Clarus 500 (Perkin Elmer) was used with a column – Restek Rtx^R– 5, (30 meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane) and an Initial oven temperature of 45^o C for 5 min Ramp 1:5^o C / min to 280^o C, and 280^o C for 15 min, Injector temperature of 280^o C, injection volume of 1.0 µl, and run time 60 min.

2.3 Preparation of Extracts

The seeds were depulped from the fruits and were washed properly under tap water and shade dried at room temperature **(Shlini**

& Siddalinga Murthy 2011). The seeds were then made into flakes which is then defatted using hexane (1:3) and the defatted flakes were dried at 60° C and powdered to obtain the defatted seed powder **(Shlini & Siddalinga Murthy 2016).**

2.4 Extraction of defatted seed powder different aqueous solvent systems

5% extract was prepared using 50mM Tris- HCl buffer pH 8.5, 50mM Acetate buffer pH 5.0, 50mM phosphate buffer pH 7.0, 0.1M sodium chloride, various concentration of sodium hydroxide (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 M NaOH) and distilled water. The powder was extracted in each of the solutions for 30 mins and centrifuged at 10,000 rpm for 20 mins. The pellets were discarded and the supernatants obtained were used for the estimation of phenolics, proteins and antioxidants.

2.5 Extraction of defatted seed powder using different organic solvents

10% extract of defatted seed powder was prepared using 80% aqueous solutions of methanol, ethanol and acetone. The sample was extracted for 30 mins, centrifuged at 10,000 rpm for 30 mins. The supernatants were collected and the residues obtained were re extracted with five volumes of respective solvents and the supernatants were pooled and evaporated to dryness. The residue obtained after evaporation was dissolved in 10 ml distilled water and made up to 25 ml. The solutions were used for the estimation of antioxidants, phenolics and proteins.

2.6 Extraction of defatted seed powder using a combination of alkali and alcohol

A 10% extract of defatted seed powder was prepared using different concentrations of ethanol (20%, 40%, 60%, and 80%) in 0.1M NaOH. The sample was extracted for 30 mins, centrifuged at 10,000 rpm for 30 mins. The supernatants were collected and the residues obtained were re-extracted with five volumes of respective solvents and the supernatants were pooled and evaporated to dryness. The residue obtained after evaporation was dissolved in 10 ml distilled water and made up to 25 ml. The solutions were used for the estimation of antioxidants, phenolics and proteins.

3. PROCEDURE:

3.1 Estimation of antioxidants:

Reducing Power Assay;

The total reducing power of the extract was determined according to the method of **(Hinneburg *et. al.*, 2006)** with slight modifications. 0.1ml of the extract was mixed with 0.9ml of distilled water and 0.5 ml of 1% potassium ferricyanide. After incubating the mixture at 50°C for 20 minutes 0.5ml of 10% trichloroacetic acid is added. This was then centrifuged at 5000 rpm for 10 minutes. 0.1 ml of the supernatant was collected and 0.1 ml of distilled water was added. 1 ml of 0.1% $FeCl_3$ was added and the absorbance measured at 700 nm. Higher the absorbance of the reaction mixture greater the reducing power. Antioxidant activity is expressed as ascorbic acid equivalents in gm/100gm dry weight of the sample.

3.2 Estimation of Phenolics:

The Total Soluble Phenolics in extracts were determined according to the Folin- Ciocalteau method with slight modifications. 0.1 ml of extract was introduced in to the test tubes. 7.9 ml of distilled water and 0.5 ml of Folin Ciocalteau reagent were added and mixed thoroughly. The tubes were then allowed to stand for 3 minutes. To this add 2.0 ml of 20% sodium carbonate and allowed to stand for 60 minutes. The absorbance was read at 650 nm. The total soluble phenolics was determined as gallic acid equivalents (GAE) in gm/100gm dry weight of sample.

3.3 Estimation of Proteins:

The estimation of proteins was carried out by Lowry's method with slight modifications. The reaction mixture consists of 0.1ml of extract, 0.9 ml of buffer and 5ml of copper reagent. After 10 mins incubation, 0.5 ml of FC reagent is added and the absorbance is read at 660 nm after 30 mins. The total protein is expressed as gm/100gm dry weight of sample as Bovine Serum Albumin equivalents **[Malick & Singh 1980].**

4 GC-MS analysis:

The seed powder (1 g / 10 ml) was extracted with solvents of different polarity (hexane, methanol, ethyl acetate and water) and centrifuged for 20 mins at 10,000 rpm. The extracts (1 µl) were injected for GC – MS analysis of volatile and semi-volatile bioactive compounds. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library **(Grover & Patni 2013).**

5 RESULTS

Plants are great source of many medically useful phenolics, flavanoids, alkaloids, steroids and terpenoid derivatives which contributes to their antioxidant property. The seeds were extracted with different solvent systems to maximize the extractability of antioxidants, phenolics and proteins. Different concentrations of NaOH (0.05, 0.1, 0.2, 0.3, 0.4, 0.5M) were used for the extraction. Among the various aqueous solvents used maximum amount of protein (13.23g/100g), phenolics (6.16g/100g) and antioxidants (1.902g/100g) were extracted with 0.1M NaOH. Among the organic solvents used for extraction, 80% ethanol showed greater extractability of 7.71g/100g of protein, 1.92g/100g of phenolics and 0.907g/100g of antioxidants.

Thus a combination of alkali with various concentration of ethanol (20%, 40%, 60%, and 80%) was used for extraction. Amongst, the 40% ethanol in 0.1N NaOH showed maximum activity of 13.85g/100g of proteins, 7.37g/100g of phenolics and 1.52g/100g of antioxidants. The result indicates that ethanol at lower concentration does not drastically affect the extraction while at higher concentrations it decreases the effect of extraction.

5.1 Figures and Tables

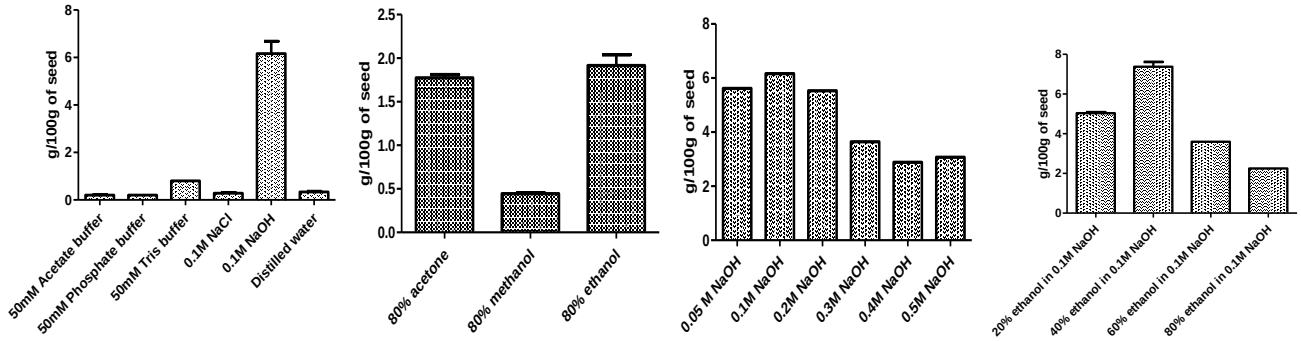

Fig 1: Phenolics profiles extracted with different solvent system

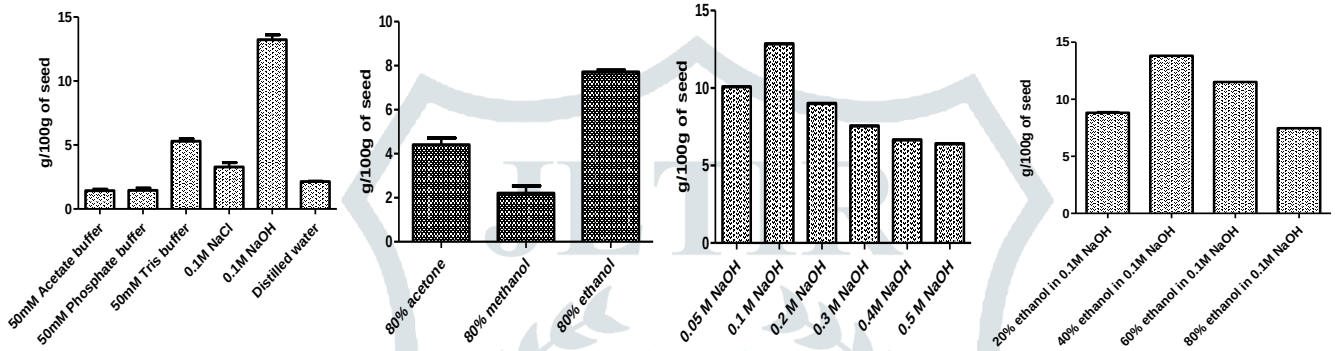

Fig 2: Proteins profiles extracted with different solvent system

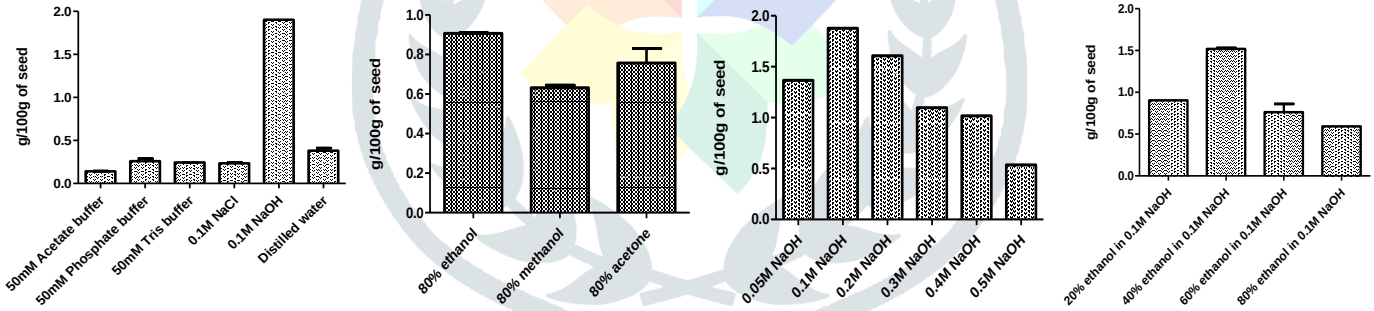

Fig 3: Antioxidant profiles extracted with different solvent system

GC-MS analysis was carried out in four different extracts like hexane, ethyl acetate, methanol and water. Among which the hexane extract showed the maximum number of 13 peaks

Table 1: Ethylacetate extract

Sl. no	*Apex RT*	*Name of compound*	*MF*	*MW*	*Area %*
1.	43.23	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.8	0.14
2.	43.48	Tetracosane	$C_{27}H_{50}$	338.6	0.16
3.	44.39	Heptacosane	$C_{27}H_{56}$	380.7	0.33
4.	44.95	Tris(2,4 di- tert- butyl phenyl) phosphate	$C_{42}H_{63}O_4$	662.9	97.72
5.	45.11	Hentriacontane	$C_{31}H_{64}$	436.8	0.68
6.	45.78	Heptacosane	$C_{27}H_{56}$	380.7	0.53
7.	46.48	7,3',4',5'- Tetra methoxy flavanone	$C_{16}H_{12}O_7$	360.4	0.44

Table 2: Hexane extract

Sl. no	*Apex R. T*	*Name of compound*	*M F*	*MW*	*Area %*
1.	8.69	Octasiloxane	$C_{16}H_{50}O_7Si_8$	507	20.39
2.	9.24	Cyclodecasiloxane, eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	741	18.22
3.	9.87	Octasiloxane S	$C_{16}H_{50}O_7Si_8$	507	1.98
4.	10.17	Spherodenon	$C_{41}H_{58}O_2$	582	0.71
5.	10.56	Cyclodecasiloxane, eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	741	21.51
6.	43.03	Aminopterin	$C_{19}H_{20}N_8O_5$	440.4	1.38
7.	43.14	Glycan Sialylated tetraose type2	-	-	2.48
8.	43.48	Tetracontane	$C_{40}H_{82}$	563.1	1.39
9.	43.81	17-Pentatriacontene	$C_{35}H_{70}$	490.9	1.02
10.	44.39	Heptacosane	$C_{27}H_{56}$	380.7	4.45
11.	45.11	Heptacosane	$C_{27}H_{56}$	380.7	7.4
12.	45.78	Heptacosane	$C_{27}H_{56}$	380.7	9.56
13.	46.48	Octacosane	$C_{28}H_{58}$	394.7	9.49

Table 3: Methanol extract

Sl. no.	*Apex RT*	*Name of compound*	*MF*	*MW*	*Area %*
1.	19.12	Benzene, 1,3-bis (1,1- dimethylethyl)	$C_{14}H_{22}$	190	0.31
2.	25.99	Dodecanoic acis,1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	638	0.37
3.	46.17	2,4, Di- tert Butyl phenol	$C_{14}H_{22}O$	206.3	39.82
4.	46.53	Octadecanoic acid,-[(1-oxododecyl)-1,2-propanediyl ester	$C_{51}H_{98}O_6$	807.3	5.04
5.	46.64	Dimyristoyl-L-α- lecithin	$C_{36}H_{72}NO_8P$	677.9	54.47

Table 4: Water extract

Sl .no.	*Apex RT*	*Name of compound*	*MF*	*MW*	*Area %*
1.	11.04	Glafenin	$C_{19}H_{17}ClN_2O_4$	372.805	32.57
2.	19.13	Benzene, 1,3- bis (1,1-dimethylethyl))	$C_{14}H_{22}$	190.33	20.44
3.	25.99	7- Acetoxy-3-formylchromone	$C_{12}H_8O_5$	232.19	46.99

6 DISCUSSIONS

The results obtained in the present study concludes that the seed extracts of *A. hirsutus* contains large amounts of phenolic compounds, exhibits high antioxidant activity and contains good protein content which can contribute for their great medicinal properties and can be used in pharmacological actions. Hence further investigation is needed to determine their biological activity. When compared to other fruits *A. hirsutus* seeds and fruits are less popular and are wasted in large quantities. So the studies can be helpful to increase the awareness of the importance of these fruits and thus can be included in diet and promote their value added products.

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