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QUALITATIVE PHYTOCHEMICAL SCREENING, ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITY STUDIES ON ETHANOLIC FLOWERS EXTRACT OF CARYOTA URENS LINN.

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ABSTRACT: The distribution of the main active principles [alkaloids, flavanoids, phenols, steroids and tannins] in the ethanolic extracts of Caryota urens flowers were screened by using various different solvents system. The anti-oxidant activity of ethanolic extracts was followed by methods of DPPH, Hydrogen per-oxide and reducing power scavenging activity. Furthermore the extract was found to possess excellent antimicrobial resistance against different pathagones of 20 bacterea and 6 fungus was determined by Agar Well diffusion method. It reveals that these flowers extracts are of therapeutic potential due to their high free-radical scavenging and antimicrobial susceptibility. The activities may be attributed to the presence of flavanoids and phenolics present in the drug.

Keywords: Caryota urens. L, Phytochemical screening, Antioxidant, Antimicrobial activity.

INTRODUCTION

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. [1]

Many familiar medications of the twenty century were developed from ancient healing traditions that treated health problems with specific plants. Folk medicine is significant source of Ayurvedic, Unani, Traditional Chinese Medicine and Medical herbalism. It incorporates crude medicinal herbs, decoctions and infusions and syrups. Folk medicine is still practiced by some vendors, hakims and vaids in remote areas and some folk preparations are of surprising high curative value. [2]

The Caryota urens is a member of the family Arecaceae (Palmae) (C. urens – Toddy, fishtail, Jaggery palm, Koonthal panai in Tamil). It is Asian species that grows from India to Burma and on the Island country of Sri Lanka. Caryota urens flowers are long plait bunches hanging down in the tree. (Fig 1).

Fig 1. Flowers of Caryota urens.

The plant pacifies vitiated pitta, hyperdipsia, arthritis and burning sensation. In Ayurveda recommends the use of C. urens for seminal weakness and urinary disorders, the juice is applied on the forehead for hemicrania. In traditional medicine porridge prepared from C. urens flower is used to treat gastric ulcer, migraine headaches, **snake bite** poisoning, as well as rheumatic swellings.Tender flowers are used as a home remedy to promote hair growth. Similarly, the root is used for tooth ailments. Palm heart also used locally as flour (destructive), especially for control of diabetes and in auruvedic medicines. [3-13].

In the present study we carried out a systematic record of the qualitative phytochemical screening in concentrated ethanolic extract of Caryota urens flowers followed by various solvent systems like n-hexane, toluene, chloroform, ethyl acetate and methanol. In order to find out the active biochemicals and phytochemicals like steroids, alkaloids, triterpenoids, Proteins, amino acids, phenolics, flavonoids, glycosides, essential oils, tannins and saponins etc…Various herbs and herbal extracts contain different phytochemicals with biological activity that can show valuable therapeutic index.The antioxidant activity of ethanolic extracts of Caryota urens flowers carried out by DPPH, Hydrogen per-oxide, and reducing power scavenging activity methods are followed. Furthermore studies of antimicrobial activity in different pathogens of 20 bacteria and 6 fungus was determined by Agar Well diffusion method or cork borer method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body.

MATERIAL AND METHODS

Collection and identification of plant material.

The flowers of C.Urens were collected from the Thanjvur district Tamilnadu in April 2009.The plant was identified and it was authenticated with vouch specimen by Rapinant Herbarium, St.Joseph'College, Trichy, Tamilnadu, India.

Phytochemical screening procedure.

Preparation of extract.

The fresh flowers of C.urens (1kg) were extracted with 80% ethanol (4x500) under reflux. The alcoholic extract was concentrated in vacou for further fractionated successively with n-hexane, (2x300), toluene (2x100), chloroform (2x400), ethyl acetate (2x600) and methanol (2x100). The solvents were then removed under reduced pressure. The florescence characteristic of ethanolic extract of Caryota urens flowers as shown in Table1.Pre-preliminary phyto-profile and qualitative phytochemical screening following the methodology of Harborne (2005) [14-15] shown in Table 2& 3.

Table 1: Florescence characteristic of ethanolic flower extract of Caryota urens.

S. No	Particulars of the treatment	Under ordinary light	Under UV light (346 nm)
1	Extract as such	Dark green	Brick red
2	Extract + 1N NaOH (aqueous)	Green	Brick red
3	Extract + 1N NaOH (alcoholic)	Dark green	Reddish green
4	Extract + 1N HCL	Blackish green	Chocolate brown
5	Extract + H_2SO_4 (1:1)	Green	Brown
6	Extract + HNO3 (1:1)	Yellow	Orange
7	Extract + Ammonia	Greenish yellow	Greenish yellow
8	Extract + Iodine	Dark brown	Brown
9	Extract + 5% FeC13	Dark–yellowish brown	Dark brown
10	Extract. + Acetic acid	Light green	Orange

Table .2. Preliminary phyto- profile for flowers extract of C urens.

	Solvent	Colour and nature of extracts	Consistency	Yield of extracts (%)
Material(1kg)	n-Hexane	green	Oily (no more crystalline material)	1.9
	Toluene	Dark green	Semi sold	0.7
	Chloroform	yellowish green	sticky	3.56
	Ethyl acetate	Wine red	Non sticky	7.87
	Methanol	brown	Non sticky	8.93

PRELIMINARY SCREENING FOR ANTIOXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ACTIVITY

To determine the DPPH assay of Sample by Gyamfi et al., Method, 2002.[16]This method free radical scavenging potential of extracts was tested against a methanolic solution of DPPH (α, α-diphenyl - β -picryl hydrazyl) antioxidants react with DPPH and convert it to α, α-diphenyl- β -picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.The change in absorbance of the samples was measured. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

Calculation

Percentage of anti - radical activity = [A - B/A] x 100

Where, 'A' is absorbance of control & 'B' is absorbance of sample.

REDUCING POWER ASSAY

To determine the reducing power assay of Sample by Yildrim et al., Method, 2001. [17] 1 ml of plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferricchloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compaired with ascorbic acid standard.

Calculation

$$\text{Percentage scavenging activity} = \frac{A_{control}\ A_{test}}{A_{control}} = \times 100$$

Where $A_{control}$ is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

Table 3: Qualitative phytochemical screening in ethanolic extract of C.urens flowers

S. NO	NAME OF THE TEST	PROCEDURE	OBSERVATION	H	T	C	ET	M
1	Alkaloids	Extact + Dragondroffs reagent Mayer's reagent Hager's reagent	Orange color White ppt Yellow ppt	- - -	- - -	- - -	- - -	+ + +
2	Glycosides	Anthrone + H2SO4+Heat	Purple or green	-	-	-	+	+
3	Carbohydrates	Extact + Molish's reagent+ conc.H2SO4 Fehling's solution A&B	Purple color Brick red color	- -	- -	- -	+ +	+ +
4	Phytosterols /triterpenoids	LiebermannTest Salkowski Test Noller's test	Bluish green Red fluorescent Pink color	+ + +	+ + +	- - -	- - -	- - -
5	Proteins & Amino acids	Biuret test Xanthoprotein test Millon's reagent test Ninhydrin test	Violet color Orange color White ppt White ppt	- - - -	- - - -	- - - -	- - - -	- - - -
6	Saponins	Extact + water+ shaking	Formation of honey comb like froth	-	-	-	-	+
7	Flavonoids	Shinoda's test Zn-HCI acid reduction test	Red colour Magenta color	- -	- -	+ -	+ +	+ +
8	Fixed oils & Fats	Spot test	Stains appear after drying	+	+	-	-	-
9	Gums/Mucilage	Extact +water	No thickening of the substance	-	-	-	-	+
10	Volatile oil			+	-	-	-	-
11	Phenolics/ Tannins	FeCl3 Extract + lead acetate+ water	Intense color Formation of white ppt	- -	- -	+ +	+ +	- +

H= n-Hexane, T = Toluene, C=Chloroform, Et =Ethyl acetate, M = Methanol. - = absent + = present

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

To determine the Hydrogen Peroxide assay of Sample by Umamaheswari and Chatterjee Method, 2008. [18] Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The inhibition was calculated. Ascorbic acid was used as standard. [19]

Calculation

$$\text{Percentage of } H_2O_2 \text{ radical scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

PRELIMINARY SCREENING FOR ANTI BACTERIAL AND ANTI-FUNGAL ACTIVITY

Test organisims

The following organisims were employed for this study as test organisims:

Bacteria

Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Shigella sp, Clostridium septicum, Clostridium tetani, Bifidobacterium bifidum, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella enteritidis, Klebsiella pneumonia, Enterobacter sp., Proteus mirabilis, Pseudomonas mutant, streptococcus sp., Proteus vulgaris, Bacillus substilis mutant, Yersinia.

Fungai

Aspergillus niger, A. flavus, A. nidulans, A. oryzae, Penicillium sp, Mucor. The bacterial and fungal pathogenic strains were obtained from the Amphigene research laboratories, Thanjavur, Tamilnadu, South India.

PREPERATION OF INOCULUM

Using sterile inoculation loop 20 pure colonies of the test organism are transferred to 5ml of sterile nutrient broth and incubated at 37 ^{0}C overnight for 18hrs. Then this bacterial culture were suspended in saline solution (0.85%Nacl) and adjusted to a turbidity of 0.5 Mac Farland standards (10^8cfu/ml). This suspension was used for preliminary screening of anti bacterial activity.

Agar well diffusion assay

The modified agar well diffusion method of Perez et al., [18] was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 **µ**L of the plants extracts and blanks (ethanol, distilled water, and hexane). The concentration of the extracts of C. Urens flowers employed was 25 **µ**g/ml. The test was carried out by triplicate. The plaques were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated by applying the expression in mm as shown in Table 4 & 5.The graphical representation of the Zone of Inhibition as shown in Fig. 2, 3, and Fig 4.

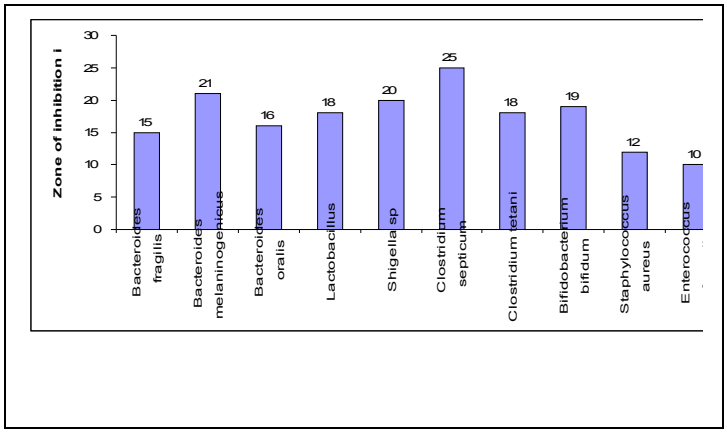

Figure 2: Anti-microbial activity in ethanolic extract of Caryota urens flowers.

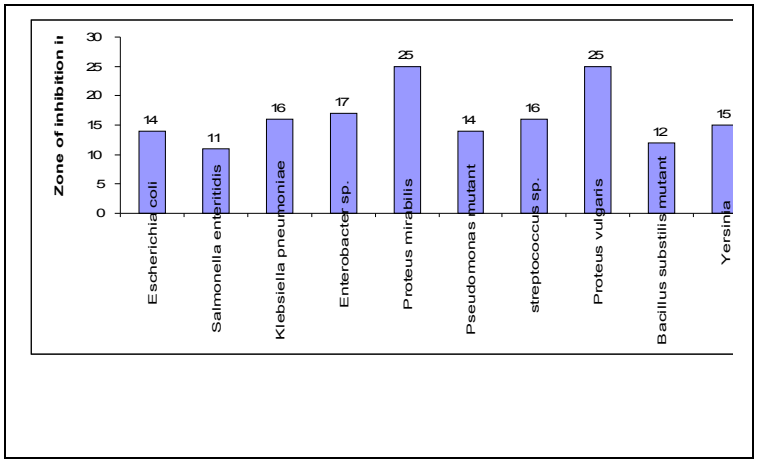

Figure 3: Anti-microbial activity in ethanolic extract of Caryota urens flowers.

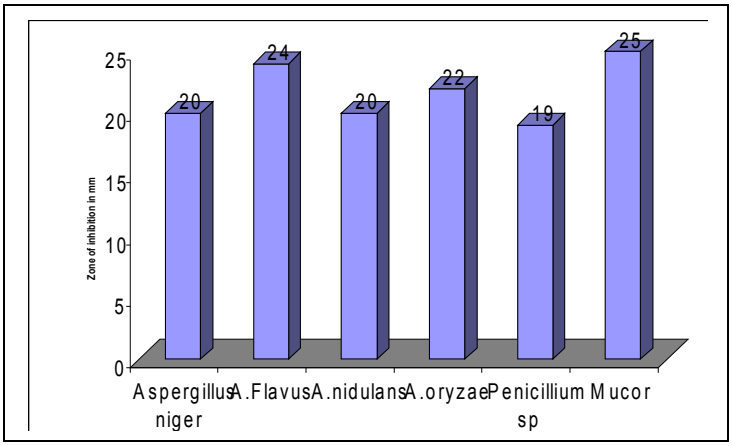

Figure 4: Anti-Fungal Activity in ethanolic extract of Caryota urens flowers.

Table: 4. Zone of Inhibition of Caryota urens extracts against selected Microorganisms

S.No	Name of Pathogens	Control	Zone of inhibition (mm)	S.No	Name of Pathogens	Control	Zone of inhibition (mm)
1	Bacteroides fragilis	0	15	11	Escherichia coli	0	14
2	Bacteroides melaninogenicus	0	21	12	Salmonella enteritidis	0	11
3	Bacteroides oralis	0	16	13	Klebsiella pneumoniae	0	16
4	Lactobacillus	0	18	14	Enterobacter sp.	0	17
5	Shigella sp	0	20	15	Proteus mirabilis	0	25
6	Clostridium septicum	0	25	16	Pseudomonas mutant	0	14
7	Clostridium tetani	0	18	17	streptococcus sp.	0	16
8	Bifidobacterium bifidum	0	19	18	Proteus vulgaris	0	25
9	Staphylococcus aureus	0	12	19	Bacillus substilis mutant	0	12
10	Enterococcus faecalis	0	10	20	Yersinia	0	15

Table: 5 Evalution of Anti-Fungal Activity in ethanolic extract of Caryota urens flowers.

S.No	Name of Species	Control	Zone of inhibition (mm)
1	Aspergillus niger	0	20
2	A. flavus	0	24
3	A.. nidulans	0	20
4	A.oryzae	0	22
5	Penicillium sp	0	19
6	Mucor	0	25

RESULTS AND DISCUSSION

In the present investigation, the active phytocomponents of C.urens flowers extract possessed steroids, alkaloids, phenolics, flavonoids, glycosides, essential oils, tannins and saponins etc., Moreover the anti-oxidant activity of C.Urens flowers extract studied on the following methods of DPPH, Hydrogen per-oxide, and reducing power scavenging activity showed the inhibition percentage is 17.30 %, 53.0 %, and 44 % respectively. The ethanolic extracts observed higher potential in Hydrogen per-oxide assay and further evaluation of antibacterial susceptibility test was conducted for isolated pathogens against the ethanolic extract of C.urens .There are 20 bacterial species and 7 fungus are used for this study which is isolated from the various organs such as Gill, Tissue, Gut ,Oral, Kidney, Urinary track and liver. The Zone of Inhibition of Clostridium septicum, Proteus mirabilis and Proteus vulgaris (25mm) is maximum with in the 20 bacteria whereas Enterococcus faecalis and Salmonella enteritidis is minimum inhibition are recorded and tabulated at a concentration of 25µg/25µl.Fungus like Mucor has of maximum and Penicillium sp minimum inhibition at a concentration of 25µg/25µl within the 6 tested pathogens. This may be due to the presence of bioactive principles like phenolics and flavonoids present in the extract of C urens flowers. Hence, the preliminary studies of herbal extract are acting as a therapatic index, especially those related to the control of resistant microbes and scavenging of free radicals.

CONCLUSION

From the above studies, it is concluded that the traditional plants may represent new sources **of** anti-microbials and anti-oxidants with stable, biologically active **components** that can establish a scientific base for the use **of** plants in modern medicine. These local ethnomedical preparations and prescriptions **of** plant sources should be scientifically evaluated and then disseminated properly and the knowledge about 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.

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REFRENCES

1. Sini K.R, Sinha B.N and Karpagavalli M (2010) Determining the antioxidant of certain medicinal plants of Attapady, India using DPPH assay, Current Botany, Vol 1. 13-16.
2. www..palace.com
3. Y.Everett (1995). Journal of Ethnobiology: Vol 15 (2) 161-171.
4. FAO (1990). Utilization of tropical foods: fruits and leaves. FAO. Rome.
5. W.D.L.Gunaratne, K.V.S.Premakumara and A.P.Heenkenda (1996). Kitul (Caryota urens L.); a popular multipurpose palm among the rural community. Proceedings of the seventh MPTS workshop 226-233.
6. H.P.M.Gunasena (eds) (1999). Multipurpose tree species in Sri Lanka: Fruits for the future. Proceedings of the 10th National workshop on multipurpose trees, Kandy, Sri Lanka. 5 Nov. 15-27.
7. J.M. Roshetko and D.O. Evans (1997). Domestication of Agroforestry trees in Southeast Asia. Yogyakarta, Indonesia.
8. R.V. Singh (1982). Fodder trees of India. Oxford & IBH Co. New Delhi, India.
9. C. Orwa, A. Mutua , R. Kindt , R. Jamnadass and A. Simons (2009). Agroforestree Database: a tree reference and selection guide version 4.0.
10. M.S.M.Sosef, L.T.Hong and S.Prawirohatmodjo (eds.) (1998). PROSEA 5 (3) Timber trees: lesser known species. Backhuys Publishers, Leiden.
11. D.H. Taylor and K.G. Macdicken (1990). Research on multipurpose tree species in Asia. Proceedings of an International Workshop held November 19-23, in Los Baños, Philippines. Winrock International Institute for Agricultural Development.
12. R.O. Williams and OBE (1949). The useful and ornamental plants in Zanzibar and Pemba. Zanzibar Protectorate.
13. K.Kadavul and A.K.Dixit (2009). Indian Journal of traditional Knowledge Vol. 8 (4) 592-597.
14. J.B.Harborne (2005) Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, 182-189.
15. Raaman,N.,(2006) Phytochemical Techniques,2nd Edition, New Indian Publishing Agency, New Delhi,19-30.
16. Gyamfi.M.A. Yonamine.M.and Aniya Y. (2002) Free radical scavenging action of medicinal herbs from Ghana Thonningia sanguinea on experimentally induced liver injuries. General. Pharmacology; 32: 661-667.
17. Yildirim.A, Mavi A. and Kara, A. (2001). Journal of Agricultural and Food Chemistry. 49, 4083-4089.
18. Umamaheswari .M. and T.K.Chatterjee (2008).Complimentary and Alternative medicines (5) 61.
19. Perez.C, Pauli.M and Bazevque.P (1990): An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis, 15:113-115.