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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF VARIOUS EXTRACTS OF *ARTABOTRYS HEXAPETALUS* FLOWERS

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ABSTRACT

Artabotrys hexapetalus belonging to family Annonaceae is widely distributed in the southern part of China, and is used in traditional Chinese medicine for the treatment of malaria and scrofula. The chloroform, methanol and aqueous extracts of *Artabotrys hexapetalus* flowers were screened for antioxidant and antimicrobial activity. The antioxidant activity is evaluated by DPPH and reducing power method. The extracts are also evaluated for total phenolic content using Folin–Ciocalteu reagent. The reducing power of extracts increases with increase in concentration of extract (5-20mg). The IC_{50} values of DPPH free radical-scavenging activity of chloroform, methanol, aqueous extracts and Gallic standards were found to be 378.73µg/ml,72.0µg/ml,118.49µg/ml and 68.75µg/ml respectively, at the concentration range of 50-250µg/ml. The total phenolic content of methanol and aqueous extracts is 22±0.9and14±0.12 mg/g plant extract expressed as an equivalent of gallic acid. Chloroform, methanol and aqueous extracts of *Artabotrys hexapetalus* flowers were screened for antibacterial activity against *Escherichia coli* (ATCC 10536), *Salmonella typhi* (ATCC 14028), *Staphylococcus aureus* (ATCC 29737), *Pseudomonas aeruginosa* ATCC (27853) and antifungal activities against *Candida albicans* and *Aspergillus niger* were compared with positive control, (Ciprofloxacin) for bacteria and (Fluconazole) for Fungi. The crude extracts exhibited significant antibacterial activity and antifungal activity. Results of the present study suggest that *A. hexapetalus* flower extract possesses strong antioxidant and antimicrobial activities.

Keywords: *Artabotrys hexapetalus*, Annonaceae, Antioxidant, Antimicrobial

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care.[1] Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. As antioxidants have been reported to prevent oxidative damage caused by free radical, it

can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers [2]. The Phytochemical substances synthesized by plants as secondary metabolites serve as the molecules of plant defense against predation by microorganisms, insects and herbivores. Furthermore, some of which involve in plant odor, pigmentation and flavor. However, several of these molecules possess medicinal
Properties.[3]

Artabotrys hexapetalus [(L.f.) Bhandari] (Annonaceae)is widely distributed in the southern part of China,and is used in traditional Chinese medicine for the treatment of malaria [4], scrofula [5] and anti-implantation/anti-fertility activity [6] .The phytochemistry of the genus Artabotry includes compounds classified as bisabolane and guaiane sesquiterpenes, steroids, aporphine and tetrahydroberberine alkaloids, and long chain hydrocarbons [7].

In this study, antioxidant activity, total phenolic content, and antimicrobial activities, of chloroform, methanol and aqueous extracts of flowers of *Artabotrys hexapetalus* were determined by reducing power and DPPH radical scavenging method, Folin–Ciocalteu method and agar diffusion method respectively.

MATERIALS AND METHODS

Chemicals

Pet. Ether, benzene, chloroform, ethanol, Methanol, Folin–Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), the other chemicals and reagents were of analytical grade.

Plant material

Flowers of *A. hexapetalus* were collected from Bangalore during July 2010.Immediately after collection; the flowers were thoroughly washed with water and dried under shade at room temperature.

Extraction

The dried flowers were coarsely powdered. 50 gm powder of flowers was subjected to successive solvent extract using chloroform, ethanol, methanol and water using a Soxhlet apparatus for 8hrs. These extracts were filtered, concentrated in Buchi rota vapor and dried under vacuum at 60^0C.

Antioxidant activity

DPPH radical scavenging activity [8]

DPPH scavenging activity was measured by the slightly modified spectrophotometric method. A solution of DPPH in methanol (6×10^{-5} M) was prepared freshly. A 3 ml aliquot of this solution was mixed with lmL of the samples at varying concentrations (50–250 µg/ml). The solutions in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. The decrease in absorbance was measured at 517 nm. The percentage inhibition of the radicals due to the antioxidant property of the extracts was calculated using the formula

$\%\text{inhibition} = A_{control} \_ A_{sample}/A_{control} \times 100\%$

Reducing Power method [9]

Accurately weighed 10 mg of the extract in 1 ml of distilled water were mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50^0C for 20 min. Following incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

Determination of total phenol content [10]

The total phenolic content of plant extracts was determined using Folin–Ciocalteu reagent. Plant extracts (100 µl) were mixed with 500 µl of the Folin–Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of Gallic acid (GAE). Total content of phenolic compounds in plant methanol extracts in Gallic acid equivalents (GAE) was calculated.

Antimicrobial activity

Antibacterial and antifungal screening [11]

Four bacteria, (two gram +ve i.e. *B.subtilis, S. aureus* and two gram-ve i.e. *E. coli, S. typhi*) and two fungi (*Candida albicans* and *Aspergillus niger*) were used in the present study. The antibacterial and antifungal activity of the crude extracts was done by agar diffusion method (cup plate method). In the agar diffusion method, nutrient agar for bacteria and Sabouraud dextrose agar for fungi were used as culture media and cavity were aseptically made over the culture plates using borer [9 mm internal diameter (i.d)]. The cavities were filled with extracts, standards and control. The plates were incubated at 37^0C for 24hrs for bacteria and 25^0C for 48 hours for fungi. The activities were determined by measuring the zone of inhibition in milli meter. The experiment was replicated two times to confirm the reproducible results. Solvent used as negative control in each time. Ciprofloxacin (1000µg/ml), for bacteria and Fluconazole (1000µg/ml) for fungi were used as Standard for comparison of the activities.

Each datum represents the mean of three different experiments in each of which two measurements were made.

RESULTS AND DISCUSSION

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, O2•− and OH• quenching assays are most commonly used for the evaluation of antioxidant activities of extracts.

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compare to other methods. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The maximum absorption of a stable DPPH radical in methanol is at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the

reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity.

Free radical-scavenging capacities of the extracts measured by DPPH assay are shown in Fig. 1.

It was observed that the percentage of DPPH scavenging is high in methanol with IC_{50} value of 72µg/ml fallowed by aqueous and chloroform 118.49 and 378.73µg/ml respectively. Inhibition values in the concentrations of 50, 100,150, 200 and 250µg/ml were, respectively given in Table no 1**.**

Reducing Power method

The reducing power of methanolic extract of *Artabotrys hexapetalus* flowers was high compare to ethanol and aqueous extract. The reducing power of the extracts was found to increase with increasing concentration of the extract.

Total phenolic content

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups. A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers. In addition, it has been reported that phenolic compounds are associated with antioxidant activity. The content of total phenolics in the extracts of *Artabotrys hexapetalus* flowers is determined using the Folin–Ciocalteu assay, calculated from regression equation of calibration curve and is expressed as gallic acid equivalents (GAE). The total phenolic content of methanol and aqueous extracts is 22±0.9and14±0.12 mg/g plant extract expressed as an equivalent of gallic acid.

Antimicrobial activity

The results suggest that aqueous extract was significant against bacteria both Gram positive *(Staphylococcus aureus* and *Pseudomonas aeruginosa*) and Gram negative(*Escherichia coli* and *Salmonella typhi*)organisms. It was also effective against the fungi (*Candida albicans* and *Aspergillus niger).* But the chloroform extract showed significant activity against the fungi but less effective against bacteria. Methanolic extract possess good activity against both bacteria and fungi.

TABLE 1: PERCENTAGE SCAVENGING ACTIVITY OF DIFFERENT EXTRACT IN DDPH ASSAY METHOD.

Extract	Percentage scavenging of extracts at different concentration(µg/ml)					IC_{50}value (µg/ml)
	50	100	150	200	250	
Chloroform	8.84	14.7	15.8	25.59	30.84	378.73
Methanol	51.35	60.92	72.71	81.24	93.97	72.00
Aqueous	16.7	40.61	57.57	68.67	83.77	118.49

TABLE 2: REDUCING POWER OF VARIOUS EXTRACT

SI NO	Concentration (µg/ml)	Absorbance at 700nm			
		chloroform	methanol	aqueous	Gallic acid
1.	5	0.325	2.12	2.01	2.24
2.	10	0.543	2.246	2.13	2.382
3.	15	0.663	2.331	2.19	2.523
4.	20	0.786	2.463	2.203	2.635

TABLE 3: THE ZONE OF INHIBITION FOR CHLOROFORM, METHANOL AND AQUEOUS EXTRACTS OF *ARTABOTRYS HEXAPETALUS* FLOWERS AGAINST DIFFERENT ORGANISMS.

SL. No.	**Name of the Drug**	***E.coli Mm****	***S.aureus** mm*	***P.aerogenosa** mm*	***S.typhii** mm*	***c.albicans** mm*	***A.nigrus** mm*
1.	Chloroform extract	5±0.02	9±0.25	10±0.01	7±0.09	10±0.98	9±0.67
2.	Methanol extract	12±0.12	15±0.07	8±0.023	10±0.02	10±0.02	8±0.95
3.	Aqueous extract	18±0.03	17±0.11	18±0.12	19±0.03	9±0.23	9±0.25
4.	Ciprofloxacin	20±0.09	20±0.09	20±0.08	20±0.09	----	----
5.	Flucanozole	---	----	----	----	10±0.01	10±0.03

Data represents an average of three determinations.

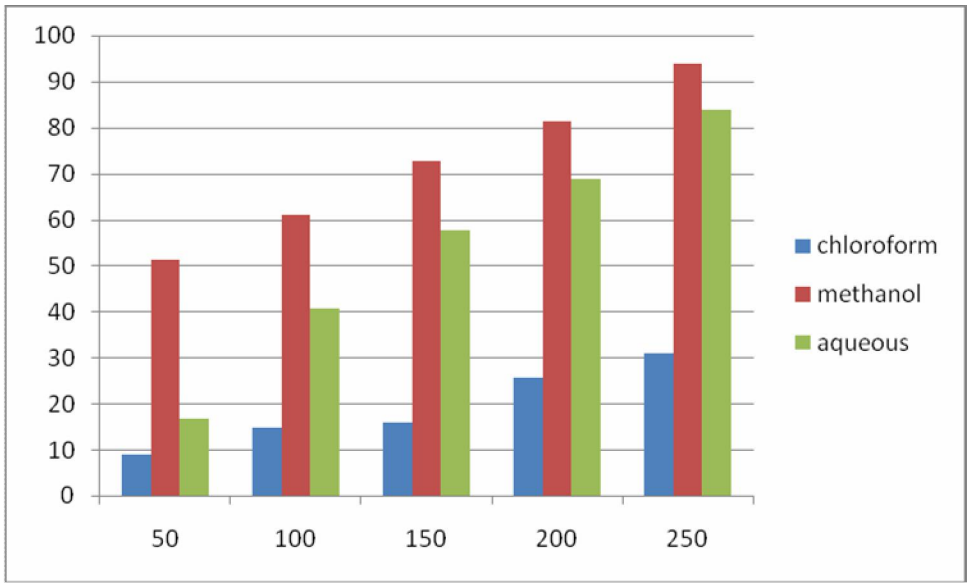

Figure 1
Free radical-scavenging capacities of the extract measured by DPPH method
X – Axis: concentration of various extracts. Y–Axis: percentage of free radical-scavenging

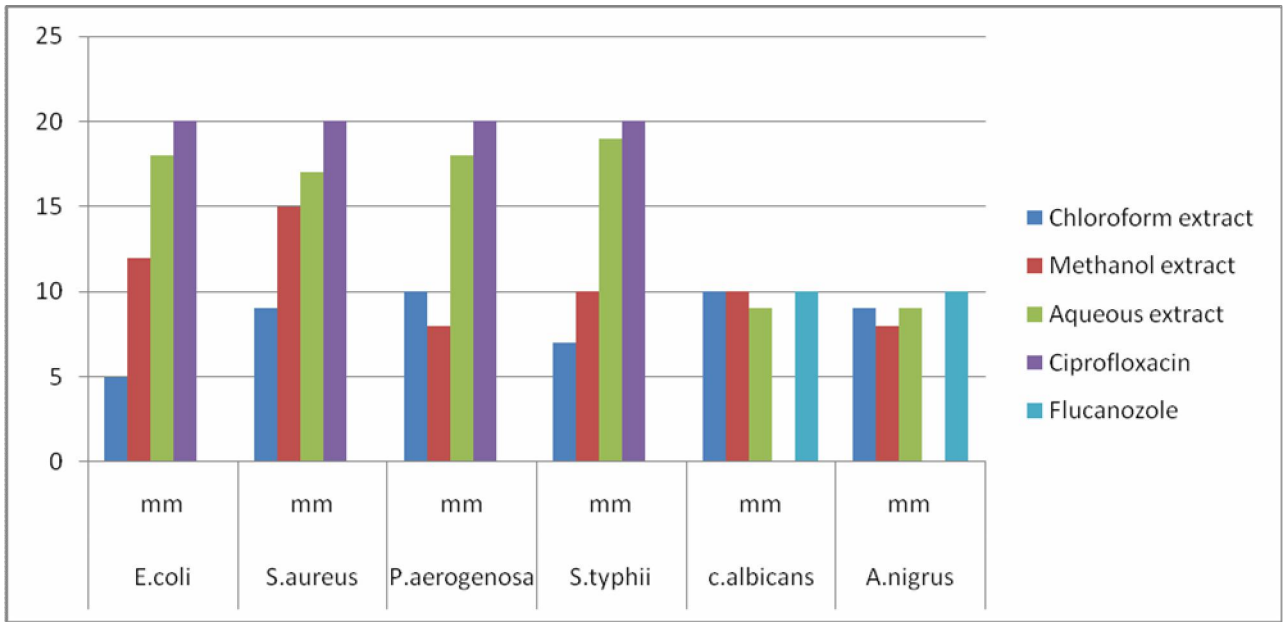

Figure 2
Graphical comparison of *in vitro* anti-bacterial activity of different extracts .
X – Axis: various extracts and positive control. Y – Axis: Zone of inhibition in mm.

CONCLUSION:

The study clearly indicates that the extracts possess significant antioxidant and antimicrobial activity. This investigation is the first report on the comparative analysis of the antimicrobial and antioxidant properties of various extract of *Artabotrys hexapetalus* flowers. These findings justify the traditional uses of this plant. Further research is necessary for elucidating the active principles.

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