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Preliminary Phytochemical Screening and Bioevaluatuion Studies of Stem Bark of *Cochlospermum gossypium*

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Abstract:

The aim of the present work was to investigate the *in vitro* antimicrobial activity, antioxidant activity and preliminary phytochemical screening of ether, chloroform, acetone, and methanol extracts of stem bark of *Cochlospermum gossypium*..In preliminary phytochemical analysis we observed carbohydrate, alkaloids, saponins, phenolic compounds, proteins. Antimicrobial activity was evaluated for nine bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*,*Sh. dysenteriae*, *Vibrio cholera*, *Bacillus subtilis* and *staphylococcus aureus*by using agar well diffusion method. Methanolic plant extract showed a maximum zone of inhibition in *staphylococcus aureus*by agar well diffusion method, acetone extract maximum inhibitory activity in *S. typhi*. The antioxidant activity of the plant extract was also determined by DPPH method using ascorbic acid as standard. *Cochlospermum gossypium,* showed 40.32% antioxidant activity as compared to standard vitamin C by DPPH method.

Keywords: traditional medicine, *Cochlospermum gossypium*, phytochemicals, antimicrobial activity, antioxidant activity.

Introduction

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern (Westh H, et al, 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow JE et al, 2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people in rural areas still rely mainly on traditional remedies. India is rich in flora and fauna. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medicinal plants. Today, Ayurvedic, Homoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Materia Medica (NarayanaRao and K. Thammanna, 1987). There is ample literature on preliminary phytochemical surveys. Plants consist of a number of biologically active compounds such as alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds (Mohamed Saleem, et al 2011). Therefore they are used for the treatment of a large number of infectious diseases. The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plants due to their nutraceutical effects (Stahelin HB, *et al*

(1991). Antioxidants is compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. In addition to their individual effects, antioxidants interact in synergistic ways and have sparing effect in which one may protect another against oxidative destruction. These justify the overwhelming interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Steinberg, D,1991).

Cochlospermum gossypium, a medicinal herb belonging to family Cochlospermaceae, is a small deciduous tree. The tree yields a gum which is known as katira. Traditionally it is used in treating cough, diarrhea, dysentery, pharyngitis, fistula, gonorrhea, trachoma and syphilis. The dried leaf and flowers are used as stimulants, antipyretic, laxative and sedative (Kirtikar K, Basu B. 1987).*Cochlospermum gossypium*stem bark paste is plastered over the bone fractured areas for over a month, stem bark and root powder is traditionally used for pregnancy and ash of fruit mixed with coconut is used for the treatment of scabies (Jayathirtha MG, 2004).

Material and Methods:

Collection of Plant Materials

The stem bark of *Cochlospermum gossypium* was collected from forest of Chichpalli village near Chandrapur, Maharashtra, India. The identification was carried out in theDept of Botany, RTM Nagpur University, Nagpur. The stem bark was washed thoroughly with tap water to remove dust particles and then washed with sterile distilled water. The stem bark was again washed with mild detergent tween 20. Again it was washed with Calcium hypochlorite. Then the stem bark was dried, powdered, stored in an air tight containers and was used for the study.

Extraction Procedure

The air dried and fine powdered stem bark was extracted with solvents viz. ether, chloroform, acetone and methanol using Soxhlet extraction apparatus according to Soxhlet method where materials are extracted by repeated percolation which lasts about 6-8 hours under reflux in a specialized glassware.

Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma chemical company and HI-MEDIA Laboratories used without further purification.

Test Microorganism

The various micro organisms such Gram negative bacteria like *Escherichia coli,Pseudomonas aeruginosa , Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris,Sh. dysenteriae, Vibrio cholera*, and Gram positive bacteria like *Staphylococcus aureus* and *Bacillus subtilisare* procured from department of Microbiology, Guru Nanak College of Science, Ballarpur.

Antimicrobial Agent

The reference standard Gentamycin was procured from Chandak Chemist and Druggist, Chandrapur, Maharashtra, India.

Antimicrobial Assay

The antimicrobial activity of ether, chloroform, acetone and methanolic extracts was tested individually by using agar well diffusion methods.

Screening of anti bacterial activity was performed by well diffusion technique. The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the nine test micro organisms. The inoculum was spread evenly over plate with loop or sterile glass spreader. The inoculated plates were incubated at 37°c for 20 minutes. After incubation a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of nutrient agar medium and 100µl of the extracts was introduced in the well and incubated at 37°c for 24 hrs and the zone of inhibition was measured in millimeter (mm). Mean zone of inhibition and standard deviations were calculated.

Phytochemical screening

Small quantity of ether, chloroform, acetone and methanol extracts were dissolved and used for detection of phytochemicals such as carbohydrates, phytosterols, proteins,alkaloids, flavonoids, tannins, saponins.

Test for alkaloids: Presence of alkaloids was tested with four reagents: Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (potassium bismuth iodide solution), Hager'sreagent (saturated solution of picric acid), and Wagner's reagent (iodine and potassium iodide solution).

Test for phytosterols

Lieberman-Burchard test and Salkowski test was performed to identify the presence of phytosterols. The residue was dissolved in few drops of acetic acid and three drops of acetic anhydride was added followed by few drops of concentrated sulfuric acid. Bluish green colour was formed shows the presence of phytosterols.

Test for glycosides

The extract was hydrolyzed with HCl for few hours on hot water bath and the hydrolysate was subjected to Fehling's, Benedict's, Barfoed's tests and the result was recorded.

Test for proteins

Small quantity of extract was dissolved in few ml of water and treated with Biuret, Ninhydrin, Xanthoproteic and Millon's reagents,

Test of saponins

Foam test was conducted by diluting the extract with 20 ml of distilled and agitated in graduated cylinder 0.1cm layerof foam was formed and the result was recorded

Test for phenolic compounds

A small quantity of extract was taken in water and $FeCl_3$ test was performed to identify the presence of phenolic compound.

Assessment of In Vitro Antioxidant Activity

Free radical scavenging activity

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Methanol extract of stem bark of *Cochlospermum gossypium*was used to assess the in vitro-antioxidant activity. Antioxidant scavenging activity was studied using 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH).

Results:

The results of the phytochemical study are given in table1.The extracts showed a broad spectrum of antimicrobial activity by inhibiting the growth of test microorganisms. Methanolic extract of stem bark of *Cochlospermum gossypium*was used to assess the in vitro antioxidant activity. Antioxidant scavenging activity was studied using 1, 1-diphenyl, 2- picrylhydrazyl free radical (DPPH). Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minute of incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. Ascorbic acid was used as reference standard.

The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

The concentration of 50 mg/ml methanolic extract showed significant rate of inhibition in *S.aureus*showing 16 mm inhibition zone by agar well diffusion method same concentration showed maximum zone of inhibition (17 mm) against *Shigelladysenteriae* (Table1). In vitro antioxidant results showed that the methanolic extract of stem bark was potent in DPPH method. (Table3).

Discussion:

The stem bark of *Cochlospermum gossypium*has been used for thousands of years for its medicinal properties. It has also been used as appetite stimulant, treatment for gastrointestinal infections, gonorrhea, trachoma and syphilis. Its use for treatment of certain types of cancer and viral infections has also been reported. It is rich in a wide variety of secondary metabolites such as phytosterols, proteins, saponins, tannins and phenolswhich has been found to have in vitro antimicrobial properties. It is unclear which active ingredients are having clinical usefulness. In this connection the present study on the ether, chloroform, acetone and methanol extracts was conducted to evaluate the antimicrobial activity of stem bark. Ether,

chloroform, acetone extracts of stem bark showed milder antimicrobial activity compared to methanolic extract, which certainly indicates that methanolic extract contain higher concentration of active antimicrobial agents such as alkaloids, saponins, tannins, which are all found in more abundant amount in of *Cochlospermum gossypium*. Preliminary results of the activity of antimicrobial agents such as plant active components, antibiotics are usually expressed in vitro as zones of inhibition around the chemical.This is in comparable to the work of Gislene*et.al.,* (2000) on the antibacterial activity of the plant extract and phytochemical on antibiotic resistance bacteria in detail; according to them any chemical that demonstrates activity with zones of inhibition of 7 mm and above is acceptable as being active, the stem bark extract of *Cochlospermum gossypium*showed 8 mm inhibition zone, therefore it contains effective antimicrobial compounds. Stem bark extracts of *Cochlospermum gossypium*showed broad spectrum antimicrobial activity since acetone and methanol extracts of stem bark have exhibited antibacterial activities against *S.aureus*,*Shigelladysenteriae.*

Table.1- Antimicrobial activity of *Cochlospermum gossypium*by agar well diffusion method

Antimicrobial activity of *C. gossypium*														
Test organism	Control DMSO+Tris Mixture	Zone of inhibition (mm) Concentration of extract mg/ml												
		Ether extract			Chloroform Extract			Acetone Extract			Methanol Extract			Gentamycin
		10	30	50	10	30	50	10	30	50	10	30	50	10
Gram positive Bacteria														
S. aureus	-	-	8	10	9	12	14	12	13	15	10	15	16	28
B. subtilis	-	-	9	11	8	10	12	10	12	14	11	13	14	25
Gram negative bacteria														
E.coli	-	-	-	-	-	-	-	-	-	9	-	-	-	22
Kleb. Pneumoniae	-	-	-	-	-	-	-	8	9	10	10	12	14	23
S. typhi	-	-	-	-	-	-	-	12	13	14	10	12	14	23
Ps. aeruginosa	-	-	-	-	-	-	-	14	15	17	11	12	13	22
P. vulgaris	-	-	-	-	8	9	10	8	10	11	8	9	10	20
Sh.dysenteriae	-	-	-	-	10	12	15	13	14	15	14	16	17	22
V. cholerae	-	-	-	-	-	-	-	9	11	12	8	9	10	20

Table. 2- Preliminary phytochemical screening of *Cochlospermum gossypium*

Sr. No	Phytochemicals	Test	Ether	Chloroform	Acetone	Methanol
1	Carbohydrate	a)Molisch's test	--	--	++	++
		b)Fehling's test	--	--	++	++
		c)Benedict's test	--	--	++	++
		d)Barfoed's test	--	--	++	++
2	Phytosterols	a)Libermannburchard test	+++	+++	+	++
		b)Salkowski test	+++	+++	+	++
3	Proteins	a)Biuret test	--	--	++	++

		b)Ninhydrin test	--	--	++	++
		c)Xanthoprotein test	--	--	++	++
		d)Millon's test	--	--	++	++
4	Alkaloids	a) Mayer's Test	--	--	++	++
		b)Drangandroff's test	--	--	+	+
		c)Hager's test	--	--	+	+
		d)Wagner's test	--	--	+	+
5	Flavonoids	a)With aqueous NaOH solution	--	--	--	--
6	Phenolic compounds	a)$FeCl_3$ test	--	--	++	++
7	Tannins	a)FeCl3 test	--	--	+++	++++
		b)Lead acetate test	--	--	+++	++++
		c)Pot. Dichromate test	--	--	+++	++++
		d)Gelatin solution test	--	--	+++	++++
8	Saponins	a) Foam test	+	+	++	++

Table. 3- Antioxidant activity of methanol extract of stem bark of *Cochlospermum gossypium*by DPPH method

Sample name	% Antioxidant Activity	IC_{50} (µg/ml)
C. gossypium	40.325	55
Vitamin C	81.78	20.9

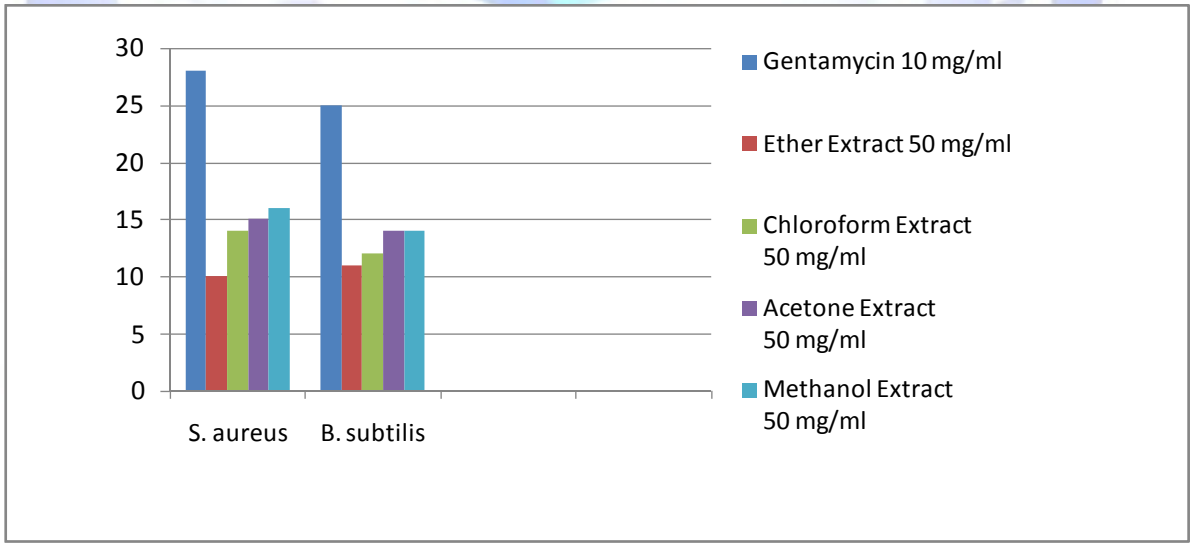

Figure. 1-

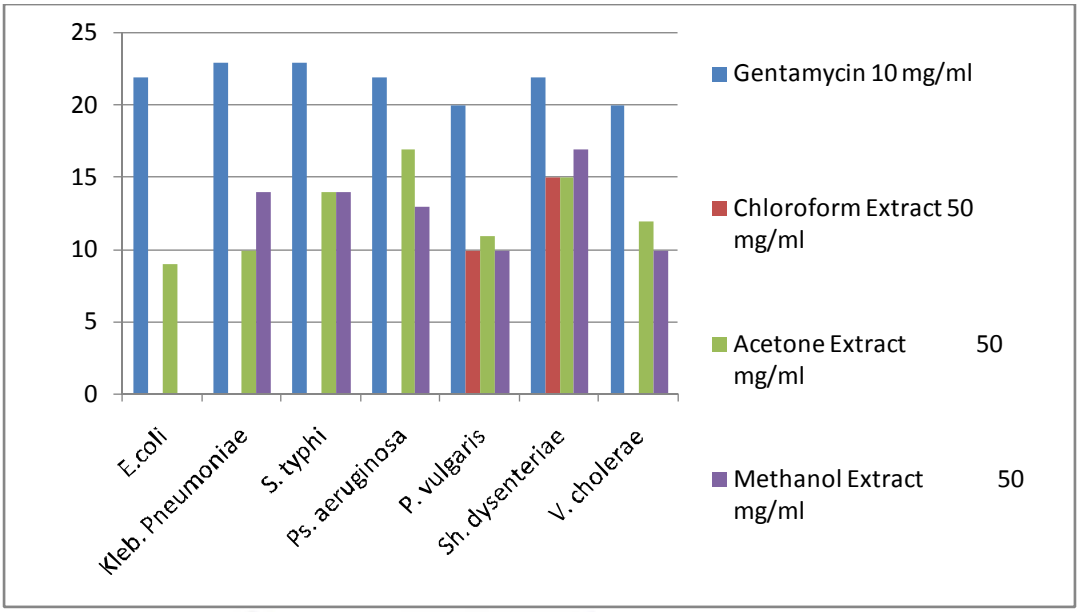

Figure. 2-

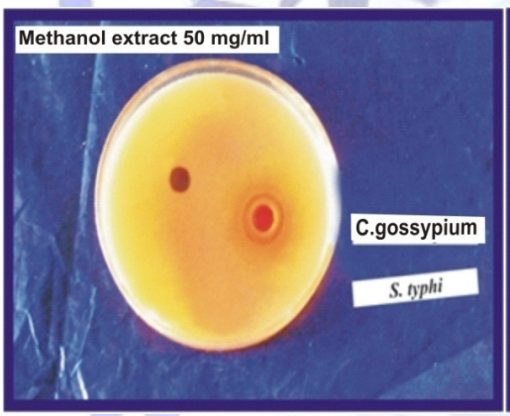

Figure. 3 (A)

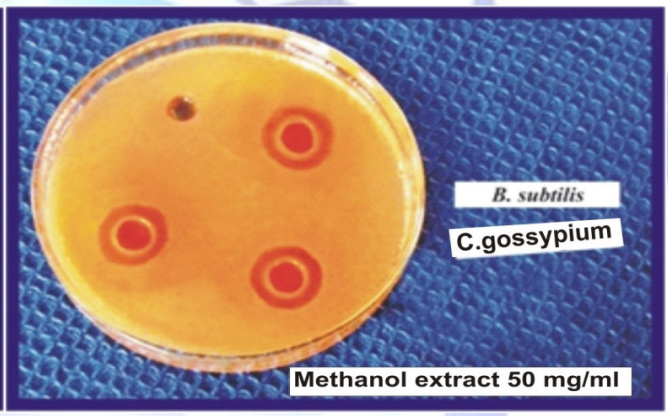

Figure.3 (B)

Figure. 1: Zone of inhibition of different extracts of *Cochlospermum gossypium*against Gram positive micro organisms by agar well method

Figure. 2: Zone of inhibition of different extracts of *Cochlospermum gossypium*against Gram negative micro organisms by agar well method

Figure. 3: (A) Zone of inhibition of different extracts of *Cochlospermum gossypium*against Gram negative micro organisms and (B) Gram positive micro organism by Agar well method

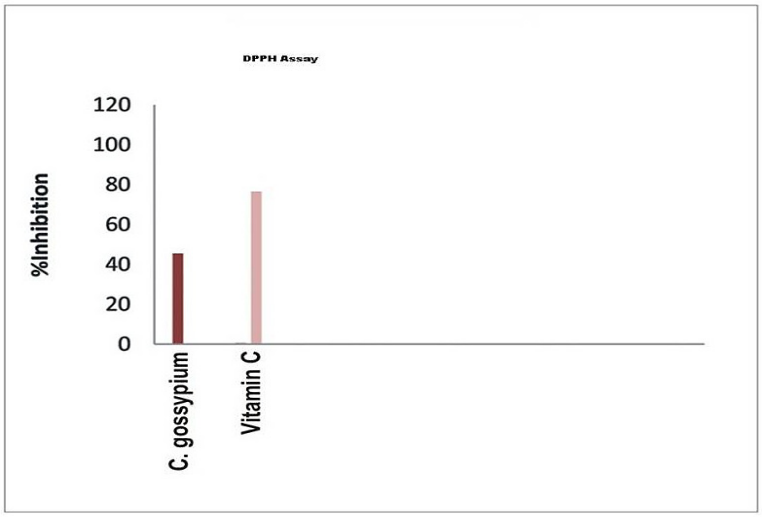

Figure.4- Antioxidant Activity of *C.gossipium*by DPPH method

Conclusion:

From our study and with previous literature survey we come to conclusion that the stem bark of *Cochlospermum gossypium*is rich inphytochemicals which has free radicals scavenging activity and strong antimicrobial activity against various microorganisms. Further studies can be made to isolate and identify the chemical nature of the antioxidant as well as antimicrobial agent present in the plant.

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