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Research Article

COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ASSAY OF FLOWER EXTRACTS OF *Butea Monosperma* AND *Cassia Fistula* AGAINST PATHOGENIC MICROBES

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ABSTRACT

Butea monosperma and Cassia fistula plants acquire the whole region in the flowering seasons and our present investigation on antimicrobial analysis and phytochemical assay was related to the concern for the amount of beautiful resource that get wasted or blown away. These plants resulted in moderate to significant antibacterial activity against the tested pathogens. Moreover the phytochemical analysis further revealed the presence of bioactive constituents to be exploited to establish drug development procedures and explore the possibility of medial healthcare to serve the society.

INTRODUCTION

Interest in medicinal plants has fascinated the scientific community to design drug using herbal products and develop new methods for healing disease (Marjorie C *et al.*)[1]. *Cassia fistula* (L.) is distributed over large area in Asia, Mexico, China, East Africa, South Africa, Mauritius and Brazil (Nair R *et al*.)[2]. The tree is basically an ornamental plant having bunches of yellow colored flowers thus named as yellow shower. Several research articles published have revealed the plant to be possessing antifertility and antimicrobial aspects on human health (Bhalerao and Kelkar, 2012)[3]. It is a mild laxative used in pregnancy containing a wax aloin and a tonic as a purgative .Mane VD *et al.* 2012[4] elucidated the plant to be effective against skin diseases, tuberculous glands, Leucoderma and pertusis. Fistucacidin pentahydroxyflavan was first extracted from the heartwood. Acetone extracts of flowers have been reported to consist of proanthocyanidin and Kaempferol. A bianthraquinone glycoside, fistulin together with kaempferol and rhein have also been isolated from ethanol extracts of *Cassia fistula* flowers. Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in the flowers (Bhalodia and Shukla, 2011)[5].*Butea monosperma* (Lam) Kuntze belongs to family *Fabaceae* which consisted of 630 genera and 18000 species. The plant is a deciduous tree having cosmopolitan distribution throughout India, Burma and Sri Lanka. Butea is commonly known as palas, palash, dhak, khakara etc and also considered as Flame of forest due to reddish orange colored flowers all over on leafless stems (Kumar and Samanta, 2012)[6]. Flowers arise at the beginning of rainy season while leaves arise at end of flowering season. *B. monosperma* is known for various biological activities including anti-inflammatory, anti-convulsant, anti-diabetic, anti-oxidant, anti-microbial, anti-diarrhoeal, hepatoprotective and many other activities (Gunakkurnu A *et al*., 2005)[7],(Kasture et al., 2012)[8]. Flowers of *B. monosperma* are used as tonic, astringent, aphrodisiac, and diuretic and prescribed in

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many forms (Sharma and Deshwal, 2011)[9]. Flowers contain various chemical constituents - butein, butin, isobutrin, coreopsin, isocoreopsin, monospermoside, isomonospermoside, aurones, chalcones, flavonoids and steroids (Perry LM, 1980)[10]. The aim of present investigation is to explore the possibility of using flower extracts instead of other plant parts viz fruits, leaves, bark which are essential nutritionally and medicinally from *Butea monosperma* and *Cassia fistula*.

MATERIALS AND METHODS

Plant and Culture Collection

The flowers of *Butea monosperma* and *Cassia fistula* used in the present study were collected in the flowering season from Kurukshetra University, Kurukshetra, Haryana, India. The flowers were identified from Botany Department of Kurukshetra University, Kurukshetra. The human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC): Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram-negative bacteria: *Escherichia coli* (MTCC-5704), *P. aerginosa* (MTCC-2295) and Gram-positive bacteria: *S. aureus* (MTCC-3160) and one fungal strain of *C. albicans* (MTCC-3017).

Preparation of Plant Extracts

Preparation of Butea flower Extract

The fully grown flowers of *Butea monosperma* were collected and taken care for its freshness, healthy and free from any deformation. These flowers were dried at room temperature then blended into powder. The 20 gm of powder was soaked in 100 ml of petroleum ether, methanol, ethanol, chloroform and aqueous extracts (cold and hot) and incubated for 72 hr at room temperature. The extracts were filtered with Whatman filter paper No-1. The solvent was evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C.

Preparation of Cassia Flower Extract

The flowers were handpicked from tree itself and shade dried under lab conditions and grounded into fine powder. The 20 gm of this powder was soaked in 100 ml of Petroleum ether, methanol, ethanol, chloroform and aqueous extracts, and incubated for 72 hr. at room temperature. The extract were filtered with Whatman filter paper No-1.The extra solvent from the filtrate was evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C.

Antimicrobial Activity of Plant Extracts

The antimicrobial activities of different solvent extracts were evaluated by agar well diffusion assay (Pereze C *et al*. 1990)[11]. The microbial inoculums were spread uniformly on surface of Mueller Hinton Agar (MHA) plates. A well of about 6.0 mm diameter was aseptically punctured using a sterile cork borer. The cut agar was carefully removed by the use of sterile forceps. DMSO was used as a negative control. The petriplates were kept in laminar for 30 minutes for pre-diffusion to occur then petriplates were incubated overnight at 37 °C for 24 hr. The antimicrobial spectrum of extract was determined in terms of inhibition zone diameters around each well. Zones were measured by using high media zone scale.

Phytochemical Screening

Preliminary phytochemical analysis was performed by using methods (Rao and Kaladhar, 2014)[12].

Test for Amino acids: 2 ml of solvent extract was mixed with 2ml ninhydrin reagent and kept in hot water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Proteins*:* 2 ml of solvent extract was mixed with 2ml biuret reagent. A violet color ring indicated the presence of peptide linkages of the molecule.

Test for Carbohydrates: 2 ml of methanolic extract was mixed with 2 drops of Molisch's reagent and shake well. Add 2 ml of concentrated sulphuric acid in the sides of the test tube .A reddish violet color ring appeared at the junction of the two layers immediately indicated the presence of carbohydrates in the sample.

Test for Alkaloids: 1 ml extract was mixed with 1% HCl and 6 drops of Mayers reagent and Dragendorf reagent. An organic precipitate indicated the presence of alkaloids in the sample.

Test for Steroids: 2 ml of acetic anhydride was mixed with 0.5 ml solvent extract and further added with 2 ml concentrated sulfuric acid. The color change from violet to blue or green indicates the presence of steroids.

Test for Cholesterol: 2ml solvent extract was mixed with 2 ml chloroform and further added with 10 drops of acetic anhydride and 2-3 drops of concentrated H_2SO_4. A red rose color change to blue green color indicated presence of cholesterol in the sample.

Test for Cardiac Glycosides: 5 ml of solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution already. This solution is further underlayered with 1ml conc. H_2SO_4. A brown ring on the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring where as the acetic acid layer, a greenish ring might form just gradually throughout thin layer

Test for Flavonoids: Aqueous extract was added with 5 ml ammonia solution and conc. H_2SO_4. A yellow coloration confirms the presence of flavonoids which disappears on standing long.

Test for Saponins: Take small amount of extract with 20 ml of distilled water. Agitate the mixture for 15 minutes in graduated cylinder. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins**:** Take 5 ml of extract with few drops of lead acetate. A yellow precipitate confirms tannins presence.

Test for Terpenoids*:* Take 2 ml solvent extract with 2 ml of chloroform and 3 ml of conc. H_2SO_4 to form a monolayer of reddish brown coloration of the interface revealed presence of terpenoids.

Test for Phlobatinins: Boil the aqueous extract with 1% aqueous HCl, deposition of red precipitate evidence the presence of phlobatinins.

Test for Fatty acids: 0. 5ml of extract was mixed with 5 ml of ether. The extract was allowed to evaporate on filter paper and dried. The appearance of transparence on filter paper confirms the fatty acids presence.

Test for Anthocyanins: 2 ml solvent extract was mixed with 2 ml of 2N NH_4Cl and ammonia. The pink red color turns to blue violet color indicated the presence of anthocyanins.

Test for Leucoanthocynins: 5 ml of aqueous extract was mixed with 5 ml of isoamyl alcohol. Upper layer appears red in color confirms leucoanthocyanins.

Test for Coumarins: 3 ml of 10% NaoH was mixed with 2 ml of aqueous extract. Formation of yellow color indicates coumarin.

Test for Phenols: 2 ml of extract was mixed with 3 ml of ethanol and a pinch of $FeCl_3$ to form greenish yellow color showing phenols presence.

Test for Quinones: 2 ml of extract was mixed with 3 ml of concentrated HCl to form green color confirm the quinines presence.

Test for Emodins: 2 ml of NH_4OH and 3ml of benzene was mixed with extract .The appearance of red color indicates presence of emodins.

RESULTS

Table 1 Inhibition zone diameters (in mm) of flower extract of *B. monosperma* in different solvents against different pathogens.

Solvent	*E.coli.*		*P. aeruginosa*		*S.aureus*		*C. albicans*	
	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
Methanol	34	40	30	32	20	22	17	18
Ethanol	34	35	15	18	15	16	14	18
Petroleum Ether	28	32	-	-	-	-	-	-
Chloroform	38	37	11	12	12	14	18	21
Aqueous (hot)	-	-	10	10	11	12	12	13
Aqueous (cold)	-	-	-	-	-	-	12	14

Table 2 Inhibition zone diameters (in mm) of flower extract of *C. fistula* in different solvents against different pathogens.

Solvent	*E.coli.*		*P. aeruginosa*		*S.aureus*		*C. albicans*	
	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
Methanol	38	39	19	23	31	35	20	23
Ethanol	37	35	18	22	28	31	21	22
Petroleum Ether	35	20	-	-	-	-	-	-
Chloroform	37	-	-	24	15	33	11	18
Aqueous (hot)	-	-	-	-	-	-	-	11
Aqueous (cold)	12	-	-	10	-	-	-	13

Table 3 Phytochemical Analysis, (+) Indicates presence of bioactive compound, (-) Indicates absence of bioactive compound

Phytoconsituent	*Cassia fistula*	*Butea monosperma*
Amino acid	-	+
Carbohydrates	+	+
Steroids	+	+
Cholesterol	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Saponins	-	+
Tannins	-	-
Terpenoids	-	-
Phlobatinins	-	+
Fatty acids	-	+
Leucoanthocyanins	-	–
Phenols	+	+
Coumarins	-	+
Quinones	-	-

DISCUSSION

The antimicrobial activity of different solvent extracts of flowers at two different concentrations (0.5 mg/ml, 1.0 mg/ml) is shown in Table 1 & 2. The zone of inhibition ranged from 10 mm to 39 mm, which is quite broad range to be expected. The activity of different extracts showed linear relationship with concentrations of extracts (mg/ml). The results revealed that methanol, ethanol and chloroform extracted most of the alkaloids which in turn resulted in bigger zone of inhibition 38 mm (0.5 mg/ml) and 39 mm (1.0 mg/ml) against *E.coli*, 31 mm (0.5 mg/ml) and 35 mm (1.0 mg/ml) against *P. aeruginosa* followed by 20 mm (0.5 mg/ml) and 23 mm (1.0 mg/ml) against *C. albicans* and 19 mm (0.5 mg/ml) and 23 mm (1.0 mg/ml) against *S. aureus*. As a preliminary research the flower exhibited bigger zone of inhibition showing significant activity against tested pathogens. Five different solvent extracts were selected which include methanol, ethanol, petroleum ether and chloroform alongwith hot and cold aqueous extract.

The cold aqueous extract of flowers was found to be active against one fungal strain (*C. albicans*) giving a zone of 12 and 14 mm at two different concentration tested (0.5 mg/ml and 1.0 mg/ml) respectively, while the hot aqueous extract resulted in a zone of 12 and 13 mm against *C. albicans* followed by a zone of 11 and 12 mm against *S. aureus* followed by a zone of 10 mm zone of inhibition against *P. aeruginosa*. Although no zone of inhibition was observed against *E.coli*. The result observed validated the concept of using cold and hot aquoes extract to cure infections of skin and decoction prepared in hot aqueous extract to be used to cure ailments. Extracts prepared using petroleum ether solvent was found to be active only against *E.coli* resulting in a zone of 28 and 32 mm at two different concentration tested 0.5 mg/ml and 1.0 mg/ml respectively. While no activity was reported against *P. aeruginosa* and *S. aureus* and *C. albicans*.This clearly demonstrate the importance of different solvents to be used against selected bacteria. Similar results were reported in *C. auriculata* (Samy and Ignachimuthu, 2000)[13] exhibiting significant antimicrobial activity against *E. coli* and *S. aureus* at a concentration of 6 mg/ml which is a higher concentration than present investigation. These results were in constrast to the earlier report where only methanolic extracts have been found to be more effective as compared to other solvents. Chauhan N *et. al,* 2011[14] used petroleum ether, chloroform, ethyl acetate and methanol as organic solvents to prepare plant extracts in *Cassia fistula* against *S. aureus*, *S. epidermidis*, *E. coli* and *K. pneumonia*.

In comparison to aqueous and petroleum ether extracts, the chloroform extract resulted in bigger zone of inhibition of 37 and 38 mm at two different concentration 0.5 mg/ml and 1.0 mg/ml respectively against *E. coli*, while the zone was comparable to hot and cold aqueous extract giving zone of 11 and 12 mm against *P. aeruginosa*, 12 and 14 mm against *S.aureus* and 18 and 21 mm against *C. albicans*. Muthukumar B *et. al,* 2014 [15]evaluated antimicrobial analysis by using leaf extracts of *Wedellia caledulacea* Less (*Asteraceae*) and reported the potential of hot and cold aqueous extracts along with ethanolic extracts using disc diffusion assay. As in India most of the decoctions are prepared in water so the finding needs to explore the viability of organic solvents in preparation of extracts but the ethanolic extracts of plant were observed to

be more effective than the hot and cold aqueous extracts which depicts the importance of organic solvents to be employed for extract preparations in antimicrobial analysis. Nair R *et. al*, 2005[2] reported the similar findings in preparing extracts using different solvent extracts.

In comparison to methanol, ethanol extracts of plant exhibited different zone of inhibition with marked difference at two different concentrations of 0.5 mg/ml and 1.0 mg/ml resulting in a zone of 34 and 35 mm against *E. coli* giving a zone of 15 and 18 mm against *P. aeruginosa*, 15 and 16 mm against *S. aureus* and 14 and 18 mm against *C. albicans*. Ethanol extract found to be more effective against *E. coli* giving a bigger zone of inhibition which was comparable to chloroform extracts.

Most of the literature finding revealed methanol to be best extracting solvent as the solvent have the capability to extract all the secondary metabolites from plant and our results well corroborate with the previous finding. Methanolic extract at 0.5 mg/ml resulting in a zone of 34 mm against *E. coli*. While increasing the concentration to 1.0 mg/ml the zone size increased proportionately to 40 mm, which validate the importance of using methanol as extracting solvent. Similarly the methanolic extract resulted in a zone of 30 mm at 0.5 mg/ml and 32 mm at 1.0 mg/ml followed by 20 mm at 0. 5 mg/ml and 22 mm at 1.0 mg/ml against *S. aureus* followed by 17 mm at 0.5 mg/ml and 18 mm at 1.0 mg/ml against *C. albicans*. The methanolic extracts resulted in greater zone of inhibition against all tested pathogens as compared to other solvents system. The results clearly revealed the selection of solvent system for greater and stronger activity of extracts against selective pathogens. Dahake and Kamble, 2014[16] screened methanolic extracts of *Butea monosperma* against 7 Gram negative and 7 gram positive strains. For gram negative bacteria the zone of inhibition ranged from 23-28 mm with highest being for *Kleibsiella pneumonia* giving a zone of 28.7mm, while for gram positive bacteria the range varied from 20-25 mm in diameter with highest zone of 25.6 mm against *S. faecalis*. So both strains were sensitive to methanolic extracts of flowers. Singh and Sahu, 2012[17] also investigated three solvent extracts, methanol, chloroform, and acetone against gram negative *E. coli* and *P. aeruginosa* using flower extracts of *Butea monosperma*.

Petroleum ether extracts were reported to be effective only against gram negative bacteria *Ecoli*, no activity was observed against gram positive and fungal strain tested. In comparison to petroleum ether, chloroform gave selective greater zone of inhibition against *E.coli* as compared to others microbes and fungus selected. The ethanolic solvent extract resulted in pronounced activity against *E. coli* compared to other pathogens in parallel. Ahmad and Khan, 2012[18] tested 2 different concentrations of 2 mg/ml and 3 mg/ml of methanolic extracts of *Butea monosperma* flower extracts against *S. aureus, B. subtilis and E. coli*. It was observed that 2 mg/ml concentration resulted in effective antimicrobial activity in *E. coli, S. aureus and B. subtilis* while an increased concentration of 3 mg/ml was effective against *P. aeruginosa* only. Our results well corroborate with that of Poornachander and Vennela, 2014[19].

Hot aqueous extract was active *S. aureus and P. aeruginosa* and *C. albicans* but no activity was recorded against *E. coli*, which might be due to bacterial cell wall composition. Suthar AR *et. al,* 2015[20] evaluated antimicrobial activity of Butea and Cassia flower extracts against three pathogens individually as well as combined extract. The results were consistent with our investigation. Both plant extracts showed activity in range of 8.0 -12.2 mm against *B. subtilis*, but on combining the both flower extracts resulted in decreased activity showing antagonistic effect. As compared the individual flower extracts resulted in a zone of 8.0-12.5 mm against *E. coli,* while combined results gave slightly decreased activity by showing zone of 9.3 to 10.2 mm. A range of 8.0-10.5 mm was recorded against *S. aureus* but synergy between two extracts was not reported in this case. The combined extracts of flowers of both plants resulted in combined and complicated results either additive or antagonistic action. The leaves /pod/bark were collected from Melghat forest regions of Amravati district of Maharastra were tested against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. The aqueous and petroleum ether extracts prepared using leaves resulted in significant activity against *S. aureus* only but no activity was observed against other three pathogens while aqueous extract of pod resulted in good to moderate activity against *B. subtilis and P. aeruginosa*. Although traditional therapy utilized boiled infusions or decoction prepared in hot or cold aqueous solutions to heal ailments but the studies revealed that methanolic extracts are more consistent in extracting bioactive components that proves to be more effective than either hot or cold aqueous extracts. The organic extracts exhibited more activity than aqueous extracts as most of the phytochemicals have higher solubility in organic solvents compared to aqueous extracts.

Similar reports have been obtained where the flower extracts have been tested for different ailments. Kandukuri and Singara, 2009 [21]selected five flowering plants from Warangal district of Andhra Pradesh and tested the efficacy of these extracts against three selective pathogens viz. *P. aeruginosa, S. aureus and B. cereus*. Among the five flowering plants *Butea monosperma* found to be more effective in inhibiting all three pathogens tested. Moreover the methanolic and ethanolic extracts resulted in bigger zone of inhibition as compared to other solvent systems. Petroleum ether failed to extract any antimicrobial compound while aqueous extracts were not active against any of the bacteria tested. The flower extracts gave differential activity against different pathogens. Muthuselvam D , 2016[22] reported antimicrobial and antifungal activity from leaf extracts of three cassia species namely *C. alata, C. fistula and C. tora* against four bacterial and four fungal strains (which includes *E. coli and S. aureus, Proteus vulgaris and S. typhii* while *A. niger, A. flavis, C. albicans and A. alternate* at two different concentrations of 100 mg/ml and 200 mg/ml. Similar to our results moderate activity was observed using chloroform and acetone extracts while petroleum ether completely failed to extract any antimicrobial compound from plants so not an effective solvent against any bacteria. Methanolic extracts of flower of *Butea monosperma and C. auriculata* gave a zone of 4-5 mm while moderate activity 1-3 mm were observed in acetone and chloroform extracts and aqueous extract of *Hibiscus rosa* giving a zone of 2 mm against *S. aureus*. Our results confirm the potential of using flowers as antimicrobial agents in comparison to other plant parts. The flower extracts

will be further analyzed for their antifertility effects in continuation to present investigation.

Phytochemical Assay

The phytochemical analysis of these plants is shown in table 3. The present investigation phytochemical screening of methanolic extracts of butea and cassia flower extracts revealed the presence of active constituents responsible for antibacterial activity. The flower extract of butea showed the presence of amino acids, carbohydrates, steroids, cholesterol, cardiac glycosides, flavonoids, saponins, phlobatinins, fatty acids, coumarins and phenolic compounds, while the cassia flower extracts showed the presence of carbohydrates, steroids, cholesterol, cardiac glycosides, flavonoids, and phenolic compounds, while quinones, leucoanthocyanins, terpenoids, tannins, were not present in both the flower extracts.

Our results well corroborate with Bhalodia NR *et. al*, 2011[5], where the cassia flower extracts showed the presence of tannins, flavonoids, saponins steroids glycosides, anthraquinones, reducing sugars, carbohydrates, proteins and amino acids alongwith triterpenoids, while the chloroform extract of same flowers resulted in presence of phenolic compounds and anthraquinones, tannins, glycosides in higher amounts. Similar reports were reported by Dahake and Kamble, 2014[16] showing the presence of five phytochemicals out of seven screened in methanolic fractions. Lohitha P, 2010[23] showed the presence of alkaloids, saponins, tannins in methanolic fractions of bark of *Butea monosperma*. Similarly Rajput and Patil,2012[24] reported steroids, terpenoids, glycosides in chloroform fraction using methanolic leaf extracts. Olusola A et. al, 2012[25] reported different plant extracts of *Cassia siberiana* revealed different zone of inhibition which in turn depends upon the bioactive components present in plants. The methanolic extracts have been reported to result in higher antimicrobial activity which indicates the susceptibility of pathogens to different chemical constituents extracted from different plant parts. The results revealed that antimicrobial activity directly or indirectly rely on chemical phytochemical constituents identified after screening either alone or in combination. The phytochemical screening revealed the presence of most active constituents using methanol as extractant. Most of the metabolites get extracted using methanol as organic solvent which is due to solubility of plant extracts in methanol as compared to other solvent systems. These phytochemicals owes therapeutic potentials. Tannins and saponins are well known for antimicrobial activity, while flavonoids have both antibacterial and antifungal activity. In comparison to flower the leaf extracts of *Cassia tora* revealed the presence of bioactive components like flavonoids, tannins, anthraquinones and terpenoids using standard qualitative tests and chromatographic methods Evans JS, 1986[26]. Preliminary screening of phytochemicals gave positive results for phenolic compounds. The future need is to characterize and purify the essential biomolecules of flowers for drug development including health care products. The research communities have to explore the potential of alternate source and to establish pharmaceutical industries for utilizing this eminent resource which get wasted every year in the flowering season. The results of present investigation indicate that antibacterial activity varies with plant part and solvent extract concentration. The purification of these phyto constituents and determination of their respective antimicrobial potencies should be the prospect route for examination.

CONCLUSION

The present investigation demonstrates the flower resource as an alternative for antibacterial analysis. The flower extracts exhibited significant antimicrobial activity against broad spectrum of antimicrobial agents. This is a preliminary report which will be further investigated for fertility aspects and drug development to exploit this beautiful resource easily available every flowering season. The study ascertains the material available is in bulk instead of waiting for fruiting season and fruit harvest for development of new drugs. The different components of flowers could be isolated for further analysis.

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