

Biological activities of three medicinal plants from district Mirpur, AJK, Pakistan

Abdul Shakoor^{1,2*}, Gul Zaib³ and Amjad ur Rahman⁴

¹The State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, No.20 Nanxincun, Xiangshan, Beijing, China

²University of Chinese Academy of Sciences, Beijing, China

³Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

⁴Department of Plant sciences, Quaid-i-Azam University, Islamabad, Pakistan

Abstract: Present research work was aimed to investigate the biological activities i.e. antibacterial, antifungal, antioxidant, cytotoxic and antitumor activities of crude methanolic extract of *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze and *Coronopus didymus* (L.) Pers. against Gram positive strains (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative strains (*Vibrio cholera*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Agrobacterium tumefaciens*, *Escherichia coli*) were screened. Best activity was observed against *K. pneumonia* and *S. aureus* by *A. arvensis* compared with other strains. *Butea monosperma* exhibited considerable activity against *S. aureus*, *V. cholera*, *E. aerogenes* and *K. pneumonia* compared with other strains. Methanolic extract of *A. arvensis* L. inhibited fungal growth against *A. niger* up to 30.2%. *B. monosperma* inhibited the growth of *A. niger* up to 43.5% and against *A. fumigatus* 27.3%. *C. didymus* inhibited the *A. fumigatus* up to 27.3% and against *A. niger*, it inhibited 48%. Brine shrimps lethality bioassay was used to evaluate the cytotoxic activity and LD₅₀ value was calculated by using probit analysis. Potato disc bioassay was designed to screen antitumor activity and data was analyzed by one way ANOVA.

Keywords: Antimicrobial, cytotoxicity, antitumor, antioxidant, medicinal plants.

INTRODUCTION

In ancient time, plants were used to cure the number of diseases. In spite of development in pharmacological industry, developed countries people rely on plants as a source of medicines (Newman, *et al.*, 2000). There are almost 250,000 – 500,000 plant species reported yet, from these reported species only a small portion has been investigated phytochemically. Historically, pharmacological evaluation of compounds having natural and synthetic foundations have been the source of numerous curative agents. Arbitrary evaluation as an instrument in finding new biologically energetic compounds has been more helpful in the field of antibiotics (Gerhartz *et al.*, 1985).

In recent time, most pharmaceutical companies are focusing and spending their lot of time and amount to develop natural products extracted from plants (Mahmood *et al.*, 2011). This practice will produce more cost effective drugs, which are easily affordable to the common people. In spite of the cost issue, it is noticed that some pathogenic bacteria are resistant to synthetic drugs. This is widely reported in literature (Davis, 1994). Pathogenic microbes have built resistance against antibiotics, which carry adverse side effects. In recent decades, many researchers have paid attention to plants extracts and biological active compounds that have been extracted from various plants (Essawi and Srour, 2000).

The present research work was conducted to investigate the biological activities i.e. antibacterial, antifungal, antitumor, antioxidant and cytotoxicity of three highly medicinal plants: *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze and *Coronopus didymus* (L.) Pers. belong to three different families: Primulaceae, Papilionaceae and Brassicaceae, respectively. Locally these plants are frequently used as indigenous remedies by people. *A. arvensis*, with local name neeli booti, is locally used for skin acne, as it has antiseptic properties. *B. monosperma* is traditionally used to remove air from ovary. Leaves are used to treat premature ejaculation and piles. It has anthelmintic, purgative and antibacterial properties. *C. didymus* is used to cure rheumatism, bone disorders.

Literature also showed some medicinal potential reports about evaluation of a few species of family Primulaceae (Amoros *et al.*, 1987), analyzed the antiviral properties of triterpene saponin isolated from *A. arvensis*. (Tambekar and Khante 2010), analyzed different plant species including *B. monosperma* for antibacterial activities against various bacterial strains. (Coelho de Souza *et al.*, 2004), reported antimicrobial activities of the most common used plant species including *C. didymus* in Rio Grande do Sul.

MATERIALS AND METHODS

Plant material collection and extraction method

Anagallis arvensis, *B. monosperma* and *C. didymus* were collected from Kakra. Kakra village is under the Pakistan

*Corresponding author: e-mail: abdul_shakoor954@yahoo.com

administered area of Azad Kashmir. Village coordinates lie at 33°7'33"N 73°52'47"E. Plants were collected during February 2010 to May 2010. Plant materials along with voucher numbers were deposited in the Herbarium of department of plant sciences, Quaid-i-Azam University, Islamabad Pakistan. Voucher numbers are 126664; 57, 125302; 10 and 125297; 25, respectively. Flowers of *B. monosperma*, leaves of *Anagallis arvensis* and *Coronopus didymus* after collection were dried in the shade and then ground to powdered form. 100 g of powdered plant material was mixed with 1 liter of 80% methanol. Filtration was carried out after 7 days. Filtration was carried out with the help of whatman 41 paper. Next step was to perform rotary-evaporator (Brinkman rota vapor, Model # R Germany). The semisolid extract was kept for complete evaporation of methanol.

Abbreviations

DMSO (Dimethyl sulfoxide), MIC (Minimum inhibitory concentration (doxycycline)

Samples preparation

1ml Dimethyl sulphoxide (DMSO) was used to dissolve 15mg of extract. This gave stock solution (15mg). Different 8 concentrations were made. Sequence of concentrations were 15, 12.5, 10, 7.5, 5.0, 3.0, 2.0 and 1.0mg/ml. positive and negative controls were carried out by using Doxycycline and pure DMSO respectively.

Antibacterial bioassay

In this current work, gram positive and gram negative strains were used. Over all there were 7 strains, among them, two were gram positive i.e. *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC6538) and five Gram negative *Vibrio cholera* (ATCC6896), *Enterobacter aerogenes* (ATCC13048), *Klebsiella pneumonia* (MTCC618), *Agrobacterium tumefaciens*, *Escherichia coli* (ATCC15224).

To carry out antibacterial activity, commonly known method, agar well diffusion was performed to screen the antibacterial activity (Patel *et al.*, 2008). In order to check the effect of 8 concentrations, 8 holes were prepared by sterile cork borer. Over all 100 µl solution was added into each hole. Plates were put into incubator at the temperature of 37°C. After one day of incubation, the zones readings were taken.

Antifungal bioassay

To evaluate antifungal activity of crude methanolic extracts, agar tube dilution method was performed as described by (Choudary *et al.*, 1995). *Aspergillus niger* (ATCC 1015) and *Aspergillus fumigatus* (ATCC1022D).

Fungal slant tubes were kept at 28 °C incubator for seven days. Following formula was used to obtain % inhibition % inhibition of fungal growth = 100 - (Linear growth in test / Linear growth in control) × 100.

Antioxidant bioassay

We performed bioassay by following Braca *et al.*, 2001 protocol. Inhibition was determined by following (Braca *et al.*, 2001).

% scavenging DPPH free radical = 100 × (1 - AE / AD)

In the above formula AE is absorbance of solution and whereas AD is the absorbance of the DPPH solution

Brine shrimp lethality bioassay

20 g of plant extract and 2 ml of methanol was added in the extract. Initially tested at 1000, 100, 10 mg/ml; three vials were prepared at each concentration for a total of nine vials. From stock sample 500, 50, 5µl were transferred to vials parallel to 1000, 100, 10mg/ml. After 24 hours, numbers of survivors were recorded and the data was analyzed through probity analysis to determine LD₅₀ value (McLoughlin and Rogers, 1998).

Antitumor bioassay

Antitumor activity was investigated according to the procedure followed by the (McLoughlin and Roger, 1998). *Agrobacterium tumefaciens* strain was cultured in nutrient broth medium (Difco) for 48 hours at 30°C. Assay was carried out thrice and data was analyzed through one way ANOVA. Percentage inhibition was calculated by using formula:

% inhibition of fungal growth = 100 - (Linear growth in test / Linear growth in control) × 100

RESULTS

Results of antibacterial activity are presented in fig. 2, 3 and 4. Each plant extract showed considerable activity against every strain. *Coronopus didymus*, *Anagallis arvensis* and *Butea monosperma* showed modest activity against each bacterial strain. Best activity was observed against *Klebsiella pneumonia* and *Staphylococcus aureus* by *Anagallis arvensis* compared with other strains. *Butea monosperma* exhibited considerable activity against *Staphylococcus aureus*, *Vibrio cholera*, *Enterobacter aerogenes* and *Klebsiella pneumonia* compared with other strains. *Anagallis arvensis* against *Klebsiella pneumonia* showed prominent activity. At 15mg/ml *Anagallis arvensis* showed 8mm inhibition. *Coronopus didymus* was not active against bacterial strains and showed no activity against *Bacillus subtilis*, *Agrobacterium tumefaciens* and *Escherichia coli*, while activity against other strain was also not effective. These activities were dose dependent, maximum at the concentration of 15 mg/ml. *Anagallis arvensis* and *Butea monosperma* exhibited momentous activity, which provide evidence to support the ethnobotanical use of plants to cure diseases by indigenous people.

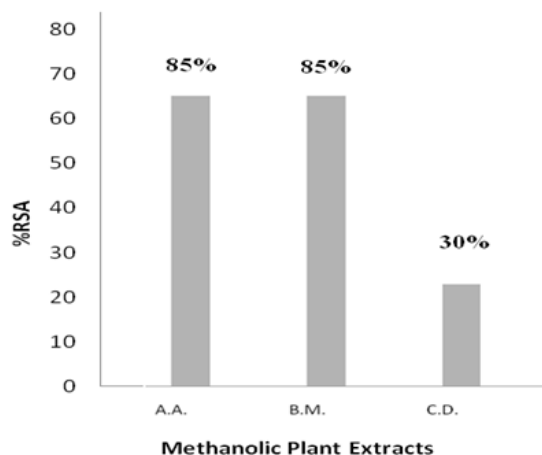


Fig. 1: Antioxidant activity of *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze, *Coronopus didymus* (L.) Pers. through DPPH

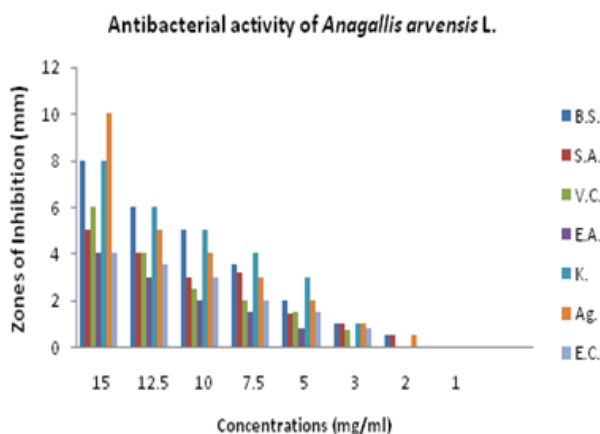


Fig. 2: Zones of inhibitions (mm) showing Antimicrobial activity of *Anagallis arvensis* L. against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Vibrio cholera* (V.C.), *Enterobacter aerogenes* (E.A.), *Klebsiella pneumonia* (K.), *Agrobacterium tumefaciens* (Ag.) and *Escherichia coli* (E.C.)

Antifungal bioassay

Results showed that the crude methanolic extract of three plants exhibited reasonable antifungal activity. *Anagallis arvensis* L. showed 68% inhibition against *Aspergillus fumigates*. Results are presented in table 1.

All these plants have potential against tested fungi. Methanolic extract of *Anagallis arvensis* L. inhibited fungal growth against *A. niger* up to 30.2%. *Butea monosperma* (Lam.) Kuntze inhibited the growth of *A. niger* up to 43.5% and against *A. fumigates* 27.35%. *Coronopus didymus* (L) Pers. inhibited the *A. fumigates* up to 27.3% and against *A. niger*, it inhibited 48%. Results of present study recommend a reasonable fine association among traditional curative use and *in vitro* antifungal activity.

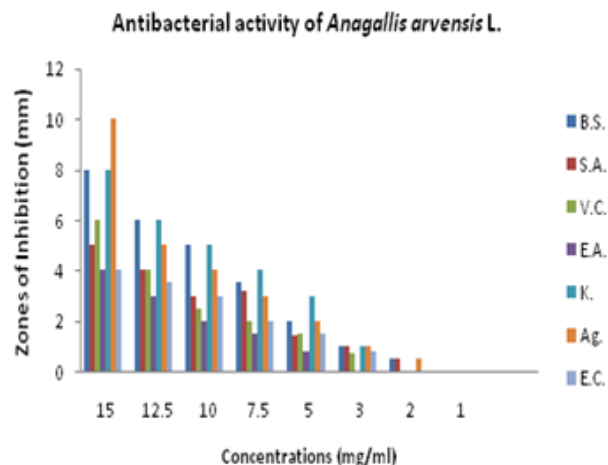


Fig. 3: Zones of inhibitions (mm) showing Antimicrobial activity of *Butea monosperma* (Lam.) Kuntze against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Vibrio cholera* (V.C.), *Enterobacter aerogenes* (E.A.), *Klebsiella pneumonia* (K.), *Agrobacterium tumefaciens* (Ag.) and *Escherichia coli* (E.C.)

Antioxidant bioassay (DPPH radical scavenging activity)

In this research work antioxidant activities of three plants were observed through DPPH free radical scavenging process. *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze, *Coronopus didymus* (L) Pers. were screened. Percentage radical activity of studied plants is shown in the fig. 1. The effectiveness of scavenging activity of crude methanolic extract of plants are in the order as *Anagallis arvensis* L = *Butea monosperma* (Lam.) Kuntze > *Coronopus didymus* (L) Pers. *Anagallis arvensis* L. and *Butea monosperma* (Lam.) Kuntze showed maximum antioxidant activity i.e. 85%, while remaining plants showed less 35% antioxidant activity.

Shrimp bioassay

Bioactive compound has toxic effects, when taken in high amount. To check the toxicity of any compound, *in vivo* lethality in a simple organism can be used as a suitable method for investigating the biologically active natural product. Brine shrimp is such type of bioassay to check the toxicity of a compound. Brine shrimp lethality bioassay is recommended protocol (Mayer *et al.*, 1982). Brine shrimp lethality method was employed to investigate the toxic effect of three medicinal plants namely *Anagallis arvensis* L, *Butea monosperma* (Lam.) Kuntze, *Coronopus didymus* (L) Pers. Results are presented in table 2. Fractions of methanolic extracts of these plants were screened to evaluate the cytotoxic potential of these plants species. Results were compiled and analyzed through probit analysis and LD₅₀ value was calculated. LD₅₀ value of *Anagallis arvensis* L was 14168 ppm that is highly insignificant (LD₅₀ >1000). *Butea monosperma* (Lam.) Kuntze result LD₅₀ 163 ppm this is significant result. This plant extract was further screened for antitumor activities. Extract of *Coronopus didymus*

Table 1: Antifungal activity of *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze, *Coronopus didymus* (L.) Pers. against *Aspergillus niger* and *Aspergillus fumigates*

Names of Plants	Fungal strains used	L.G.C. (mm)	L.G.T. (mm)	% Inhibition
<i>Anagallis arvensis</i> L.	<i>A.niger</i>	115	70	39.2
	<i>A. fumigates</i>	110	35	68
<i>Butea monosperma</i> (Lam.) Kuntze	<i>A.niger</i>	115	65	43.5
	<i>A. fumigates</i>	110	80	27.3
<i>Coronopus didymus</i> (L.) Pers	<i>A.niger</i>	115	60	48
	<i>A.fumigates</i>	110	80	27.3

Aspergillus niger – *A. niger* and *Aspergillus fumigates*- *A. fumigates*, L.G.C. (mm) = Linear Growth in Control (millimeter), L.G.T. (mm) = Linear Growth in Test (millimeter)

Table 2: Cytotoxic effect of methanolic extract of *Anagallis arvensis* L., *Butea monosperma* (Lam.) Kuntze, *Coronopus didymus* (L.) Pers. on brine shrimps (*Artemia salinia*)

Plant names	Concentration used (ppm)	No. of Shrimps used	No. of Shrimps killed	LD ₅₀ Value (ppm)
<i>Anagallis arvensis</i> L.	1000	30	21	14168
	100	30	27	
	10	30	30	
<i>Coronopus didymus</i> (L.) Pers.	1000	30	19	22100
	100	30	24	
	10	30	25	
<i>Butea monosperma</i> (Lam.) Kuntze	1000	30	10	163
	100	30	21	
	10	30	27	

(L) Pers. gave LD₅₀ value 22100 ppm (LD₅₀ > 1000). This result is again highly insignificant. These plants can be used as alternate of good drug having less toxic effects. However further research is necessary to provide better results. Brine shrimp bioassay is a first step to find anticancer drug. If a plant extract has potential to produce a number of cytotoxic secondary metabolites, it means tested plants carry potential to find anticancer drug. More investigations and research work may aid research depth to recognize bioactive compounds of tested plants.

Antitumor activity

In potato disc assay, *Agrobacterium tumefaciens* is used as the tumor causing agent, because of its unique capacity for trans- kingdom sex (Stachel and Zambryski, 1998). Results showed that *A. tumefaciens* has potential for the production of tumors. *Butea monosperma* (Lam.) Kuntze showed 66% inhibition of tumor cells. *Anagallis arvensis* L showed 50% inhibition through potato disc bioassay. *Coronopus didymus* (L) Pers. was found to be inactive, however it exhibited 8.5% inhibition rate.

Active plants could further be screened for the discovery of new antitumor agents. Results were checked through statistical analysis one way ANOVA to check the correlation among different methanolic extracts of plants. Results of one way ANOVA are presented in fig. 5. These results were further verified by LSD analysis, which showed the significant and non-significant behavior of

data. The rank of selected medicinal plants for this antitumor activity is as *Butea monosperma* (Lam.) Kuntze > *Anagallis arvensis* L > *Coronopus didymus* (L) Pers.

DISCUSSION

Pakistan has diverse vegetation. People living in rural areas are interested to use plant-based drugs. Most of the plants have no side effects and they are inexpensive.

In this current work, *Anagallis arvensis* displayed antibacterial activity against gram negative bacteria (*klebsiella pneumonia*) and *Staphylococcus aureus*. Usually gram negative strains are less susceptible, due to presence of extra membrane (Yao and Moellering, 1995). Previously Shtayeh *et al.*, 2008 showed that *Anagallis arvensis* was active against gram negative bacilli (*Proteus vulgaris*).

Furthermore, we showed that *Butea monosperma* is active against gram positive and gram negative strains. Our findings about *Butea monosperma* differ (Gurav *et al.*, 2008). They showed that *Butea monosperma* is inactive against the gram negative bacteria. Different results may be due to handling of samples and methods.

Coronopus didymus did not exhibit antibacterial activity. Study from South Brazil showed that bacterial strains were resistant against of (Coelho de souza *et al.*, 2004).

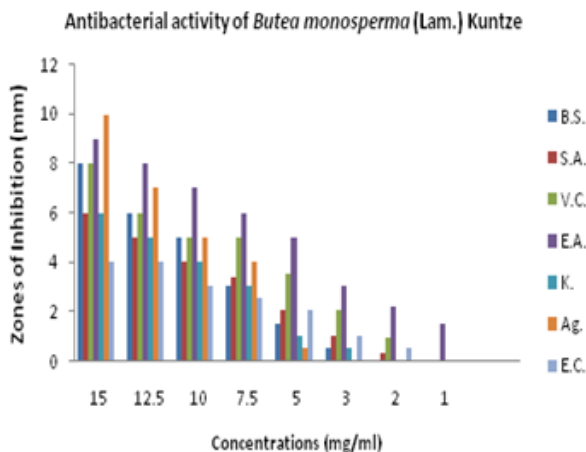


Fig. 4: Zones of inhibitions (mm) showing Antimicrobial activity of *Coronopus didymus* (L.) Pers. against *Bacillus subtilis* (B.S.), *Staphylococcus aureus* (S.A.), *Vibrio cholera* (V.C.), *Enterobacter aerogenes* (E.A.), *Klebsiella pneumonia* (K.), *Agrobacterium tumefaciens* (Ag.) and *Escherichia coli* (E.C.)

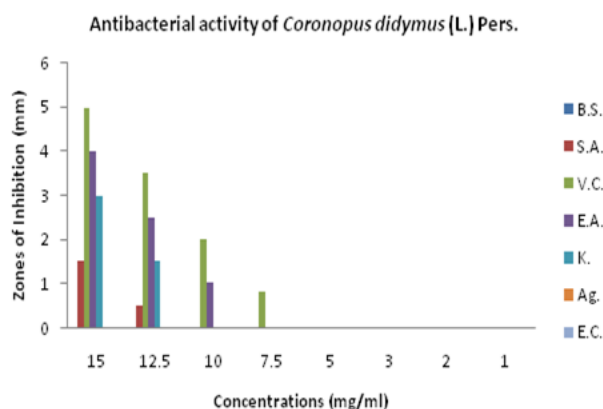


Fig. 5: Antitumor activities of *Anagallis arvensis* L. (P4), *Coronopus didymus* (L.) Pers. (P1), *Butea monosperma* (Lam.) Kuntze (P3), Control (C)

Apart from antibacterial study, our antifungal research yielded significant result. We demonstrated that *Anagallis arvensis* have high potential against tested fungal pathogens. Our results coincide with (Lopez et al., 2008). *Anagallis arvensis* also showed antidermatophytic effects (Shtayeh and Abu Ghadeib 1999). Their work illustrated the importance of above mentioned plants. They showed 90-100% inhibition rate of *Anagallis arvensis* against dermatophytes.

Most of the plants have natural antioxidant capacity and efficiency, therefore it is important to test their antioxidant potential. In continuation of that we also examined the plants. We showed that *Anagallis arvensis* and *Butea monosperma* showed maximum antioxidant activity. Previously (Prabhakar et al., 2006) displayed free radical scavenging activity of *Coronopus didymus*. They showed that *Coronopus didymus* is radio protector, wound healing

and alleviate allergies, inflammation, hepatotoxicity and hyperglycemia. However in our study, *Butea monosperma* displayed minimum antioxidant as compared to other plants studied.

Lopez et al. (2008) showed that methanolic extracts of *Anagallis arvensis* were found to inhibit the growth of *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*. *Anagallis arvensis* evidenced a higher activity as indicated in current work.

Plants produce antioxidant effect, owing to presence of flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman and Moon, 2007). A number of neurodegenerative diseases and AIDS, cancer could be cured due to presence of free radicals in plants. Present research work suggests that these screened plants have considerable amount of antioxidant activity. However it is recommended that high and deep investigation would pave and discover new antioxidant properties in the plants. The results of the present study provide a scientific validation for the popular use of medicinal plants.

ACKNOWLEDGEMENTS

Abdul Shakoor is the recipient of 2013 CAS-TWAS President's Postgraduate Fellowship.

REFERENCES

- Amoros M, Fauconnier and Girre L (1987). *In vitro* antiviral activity of a saponin from *anagallis arvensis*, primulaceae, against herpes simplex virus and poliovirus. *Antiviral Res.*, **8**: 13-25.
- Braca A, Tommasi ND, Bari LD, Pizza C, Politi M and Morelli I (2001). Antioxidant principles from *Bauhinia terapotensis*. *J. Nat. Prod.*, **64**: 892-895.
- Choudhary MI, Dure-e-Shawar, Perveen Z, Jabbar A, Ali I and Rehman A (1995). Antifungal steroidal lactones from *Withiana coagulance*. *Phytochemistry* **440**(4): 1243-1246.
- Coelho de Souza G, Haas APS, Poser GL, Schapoval EES and Elisabetsky E (2004). Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J. of Ethnoph.*, **90**: 135-143.
- Davis J (1994). Inactivation of antibiotic and the dissemination of resistance genes. *Science*, **264**: 375-382.
- Essawi T and Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. *J. of Ethno.*, **70**: 343-349.
- Gurav SS, Gulkari VD, Nandkishore J, Duragkar NJ and Arun TP (2008). Antimicrobial activity of *Butea monosperma* Lam. *Gum. IJPT.*, **7**: 21-24.
- Gerhartz W, Yamamota YS, and Campbell FT et al. (2005). Indigenous Encyclopedia of Industrial. phytotherapy for genito-urinary diseases. *Appl. Mic.*, **39**: 93-97.

- Lopez V, Akerreta S, Casanova E, Mina JG, Cavero R, and Calvo M (2008). Screening of Spanish Medicinal Plants for Antioxidant and Antifungal Activities. *Pharm Biol.* **46**(9): 602-609.
- Mahmood A, Mahmood A and Tabassum A (2011). Ethnomedicinal survey of plants from District Sialkot, Pakistan. *J. App. Pharm.*, **02**(03): 212-220.
- McLoughlin JL, and Rogers LL (1998). The use of Biological assays to evaluate Botanicals. *Drug Info. J.*, **32**: 513- 524.
- Meyer BN, Ferrighi NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL (1982). Brine shrimp: A convenient general Bioassay for active plant constituents. *Planta Medica* , **45**: 31-34.
- Newman DJ, Cragg GM and Snader KM (2000). The influence of natural products upon drug discovery. *Nat. Prod. Res.*, **17**: 215-234.
- Patel JD, Patel DK, Shrivastava A and Kumar P (2008). Screening of plant extracts used in traditional antidiarrheal medicines against pathogenic. *Escherichia coli. Scientific World*, **6**(6): 63-67.
- Prabhakar KR, Veeres VP, Vipani K, Sudheer M, Priyadarsini KI, Satish RBSS and Unnikrishnan MK (2006). Bioactivity-guided fractionation of *Coronopus didymus*: A free radical scavenging perspective. *Phytomedicine* **13**:8, 591-595.
- Rahman MAA, and Moon SS (2007). Antioxidant polyphenol glycosides from the Plant *Draba nemorosa*. *Bull. Korean Chem. Soc.*, **28**(5): 827-831.
- Stachel SE, and Zambryski PC (1998). Generic trans-kingdom sex. *Nature*, **340**: 190-191.
- Shtayeh MSA, Yaghmour RM, Faidi YR, Salem K and Al-nuri MA (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *J. of Ethnoph.*, **60**(3): 265–271.
- Shtayeh MSA, and Abu Ghdeib SI, (1999). Antifungal activity of plant extracts against dermatophytes. *Mycoses*, **42**: 665-672.
- Tambekar DH and Khante BS (2010). Evaluation of Antibacterial properties of Ethnomedicinal herbs used by Korkus in Melghat of India against Enteric pathogens. *Inter. J. of Pharma and Bio. Sc.*, **1**: 1234-1245.
- Yao J. and Moellering R (1995). Antibacterial agents. *Manual of Clinical Microbiology*. ASM, Washington, DC, pp.1281-1290.