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Research Article

# EVALUATION OF ANTIBACTERIAL POTENTIAL OF PLANT EXTRACTS USING RESAZURIN BASED MICROTITER DILUTION ASSAY

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**ABSTRACT**

Objective: Antimicrobial potential of ten medicinal plants i.e. *Picrorhiza kurroa*, *Datura metel*, *Acacia catechu*, *Cissus quadrangularis*, *Cassia tora*, *Berberis aristata, Pongamia pinnata, Emblica officinalis, Saraca asoca* and *Tinospora cordifolia* was evaluated against six bacterial strains i.e. *Bacillus subtilis*, *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

Methods: Plant extracts were prepared by using Soxhlet extraction. Five extract from each plant were prepared using five solvents on the basis of increasing polarity. Minimum inhibitory concentration of extracts was determined by resazurin based microtiter dilution assay.

Results: Percent yields of petroleum ether and chloroform extracts of plant leaves was found to be in the range of 0.80 - 2.98%. Percent yields of acetone, methanol and water extract were found to ranging from 2.87- 5.77%. *S. asoca* extracts were found to be endowed with highest antimicrobial activity out of the ten plants used in the study. Leaves water extract of *S. asoca* showed lowest minimum inhibitory concentration (0.15 mg/mL) against *Pseudomonas aeruginosa.*

Conclusion: It was observed that leave water extracts of *S. asoca* could be potential reservoir of bioactive compounds. Post treatment analysis of proteome of test micro-organisms could explore potential anti-bacterial targets.

**Keywords:** Resazurin, Microtiter dilution assay.

## INTRODUCTION

In the era of modernization and changed environmental conditions man frequently encounters pathogenic microorganisms causing infectious diseases. The indiscriminate use of commercially available antibiotics for the treatment of infectious diseases developed multiple drug resistance in the microorganisms, putting new challenge before the drug industries for identification of new efficient antimicrobial compounds. Herbal drugs therapy is regarded as an important alternate, leading the researchers to focus and evaluate the traditionally recommended medicinal plants for their efficacy in various disease conditions [1].

Medicinal plants are major part of new pharmaceuticals and health care products. Due to the availability of medicinal plants throughout the world, herbal drugs are being used by 75-80% of world population, especially in developing countries. As reported by World Health Organization (WHO), traditional medicinal plants are the best reservoirs to develop newer pharmaceuticals [2]. Medicinal plants are renewable sources therefore farmers get encouraged to include them in traditional agriculture [3]. India has highly diverse vegetation and herbal plants which are rich source of bioactive compounds. To ensure the commercial medicinal potential of plants depicted in Ayurveda, the antimicrobial potential need to be evaluated against present day pathogens according to new parameters to ensure there efficacy and reliability. In the present study, ten plants were shortlisted on the basis of literature survey and there extracts were evaluated for antimicrobial potential against six bacterial strains (Table 1). Minimum inhibitory concentration (MIC) of plant extracts was determined using microbroth dilution assay.

**Table 1: Traditional and Medicinal properties of Plants used in the study**

| S. No. | Botanical Name | Local name | Medicinal property | Reference |
|---|---|---|---|---|
| 1. | *Picrorhiza kurroa* | Katuka, Kutki and Hellbore | Skin, urinary tract, diarrheal infections, gastrointestinal infections, antioxidant, antidiabetic, anti-allergic, anti-hyperglycaemic, hepatoprotective, immunostimulating, anti-cancer, and anti-inflammatory. | [5-7] |
| 2. | *Datura metel* | Dhatura | Antibacterial, antioxidant, herbicidal, analgesic, anesthetic, antispasmodic, antitussive, bronchodilator and hallucinogenic. | [8-10] |
| 3. | *Acacia catechu* | Black Cutch, Khair and Katha | Sore throats and diarrhoea, high blood pressure, dysentery, colitis, gastric problems, bronchial asthma, cough, leucorrhoea, immunomodulatory, antipyretic, antimycotic. | [11-14] |
| 4. | *Cissus quadrangularis* | Hadjod, Asthisamdhani | Bone fracture healing activity, antiulcer, antiosteoporotic effect, antibacterial, antiprotozoal. | [15-19] |
| 5. | *Cassia tora* | Chakunda | Hepatoprotective, antigenotoxic, hypotensive, antibacterial and antifungal. | [20-24] |
| 6. | *Berberis aristata* | Indian barberry, Daru haldi, Chitra | Ear infection, antibacterial, Antifungal, anti-inflammatory, analgesic, antipyretic and anticancer. | [25-27] |
| 7. | *Pongamia pinnata* | Karanja, Karanj | Antiinflammatory, anti-plasmodial, anti-nonciceptive, anti-hyperglycaemics, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic, CNS depressant activity, antioxidant and antibacterial, antifungal | [28-30] |
| 8. | *Emblica officinalis* | AmLa, Amalaka, Aavalaa, AmLaki and Indian gooseberry | Anti-viral, antibacterial, anti-cancer, anti-allergy, and anti-mutagenic. | [31] |
| 9. | *Saraca asoca* | Ashok briksh, Ashoka | Antioxidant, antibacterial, anticancer, oxytocic and antilarval. | [32-37] |
| 10. | *Tinospora cordifolia* | Gulancha, Giloy | Anti-HIV, anti-parkinson's disease, Anti-stress, anti-inflammatory, antibacterial. | [38-40] |

## MATERIALS AND METHODS

### Plant material

*Picrorhiza kurroa*, *Datura metel*, *Acacia catechu*, *Cissus quadrangularis*, *Cassia tora*, *Berberis aristata, Pongamia pinnata, Emblica officinalis, Saraca asoca* and *Tinospora cordifolia* were selected for study (Table 1) and identified by Department of Botany, Maharshi Dayanand University, Rohtak (India). Cross authentication of selected plant was done with the help of flora of Haryana [4]. The herbarium specimens were preserved at Centre for Biotechnology, M D University, Rohtak.

### Preparation of Crude Extract

Leaves of ten different medicinal plants were collected and air dried by keeping in shade for 3 weeks. Afterward, the plant materials were transferred to oven at 40°C for 20-24 hrs. The properly dried plant leaves were grinded to fine powder with the help of electronic grinder. Sixty gram leaves powder of each plant was extracted by Soxhlet's method. For crude extraction, five solvents (300 mL each) were used in ascending order of polarity i.e. petroleum ether, chloroform, acetone, methanol and water. The leaf powder extracts were filtered twice, firstly filtered under the vacuum through a double layer of Whatman filter paper (No. 3 and No. 1) and secondly through a single sheet of Whatman No. 1 filter paper under gravity. The clear supernatants were subjected to vacuum distillation at 30-35 °C using a Buichi Rotary Evaporator for removing the solvent. The remaining residues were stored in refrigerator till further use.

### Micro-Organisms and reference strain

Six Microbial Type Culture Collection (MTCC) registered bacterial isolates were obtained from Institute of Microbial Technology, Chandigarh (Table 2) and used for the evaluation of antibacterial activity of crude plant extract.

**Table 2: Six standard strains of bacteria given below were used in the study.**

| Name | Types | Strain type |
|---|---|---|
| *Escherichia coli* | Gram Negative | MTCC433 |
| *Salmonella typhi* | Gram Negative | MTCC531 |
| *Staphylococcus aureus* | Gram Positive | MTCC9011 |
| *Bacillus subtilis* | Gram Positive | MTCC441 |
| *Pseudomonas aerguinosa* | Gram Negative | MTCC7925 |
| *Klebsiella pneumoniae* | Gram Negative | MTCC3384 |

### Preparation of bacterial culture

Using aseptic techniques, six 100 mL bottles of Luria broth were inoculated with six bacterial strains (each bottle contain only one type of bacteria) and kept in the incubator overnight for 12-18 h at 35° C. After incubation, all bacterial cultures were centrifuged at 4000 rpm for 5 min. Supernatants were discarded and pellets were re-suspended in 20 mL of sterile normal saline and re-centrifuged at 4000 rpm for 5 min. The pellet was dissolved in 20 mL of sterile normal saline and was labelled as bacterial solution. Optical densities (OD) of the bacterial solutions were measured at 600 nm and serial dilutions were made until the OD was in the range of 0.5-1.0. The viability graph was used to calculate the actual number of colony forming units. The dilution factor was calculated and the dilution was performed to obtain a final concentration of 5 x $10^6$ CFU/mL [41].

### Preparation of Resazurin Dye Solution (RDS)

Resazurin dye (300 mg) was dissolved in 40 mL sterile water. Vortex mixer was used to homogenize the solution**.** This solution was then referred as Resazurin dye solution. Resazurin is an oxidation-reduction indicator used for the evaluation of cell growth, particularly in various cytotoxicity assays [42]. It is purple non-fluorescent and non-toxic dye becomes pink and fluorescent when reduced to resorufin by oxidation reduction within viable cells. Resorufin is further reduced to hydroresorufin (uncoloured). Resazurin reduction test has been used from decades to demonstrate bacterial and yeast contamination of milk [43].

### Resazurin based Microtiter Dilution Assay (RMDA)

Under aseptic conditions, 96 well microtitre plates (Tarson) were used for Resazurin based Microtitre Dilution Assay. The first row of microtiter plate was filled with 100 µl of test materials in 10% (v/v) DMSO or sterile water. All the wells of microtitre plates were filled with 100 µl of nutrient broth. Two fold serial dilution (through out the column) was achieved by starting transferring 100 µl test material from first row to the subsequent wells in the next row of the same column and so that each well has 100 µl of test material in serially descending concentrations. 10 µl of resazurin solution as indicator was added in each well. Finally, a volume of 10 µl was taken from bacterial suspension and then added to each well to achieve a final concentration of $5\times10^6$ CFU/mL. To avoid the dehydration of bacterial culture, each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each microtitre plate had a set of 3 controls: (a) a column with Streptomycin as positive control, (b) a column with all solutions with the exception of the test extract and (c) a column with all solutions except bacterial solution replaced by 10 µl of nutrient broth. The plates were incubated in temperature controlled incubator at 37° C for 24 h. The colour change in the well was then observed visually. Any colour change observed from purple to pink or colourless was taken as positive. The lowest concentration of plant leaf extract at which colour change occurred was recorded as the MIC value. All the experiments were performed in triplicates. The average values were calculated for the MIC of test material.

## RESULT AND DISCUSSION

### Percentage Yield

Different plant extracts were prepared from selected ten plants using five solvents of different polarity. Percent extract yield of these plants varies from 0.80 to 5.77% (Table 3). In most of the cases the amount of residue extracted with acetone, methanol and water is higher than other two solvents. Percent yields of petroleum ether and chloroform extracts of plant leaves was found to be in the range of 0.80 - 2.98%. Percent yields of acetone, methanol and water extract were found to ranging from 2.87- 5.77% (Table 3).

**Table 3: Percent (%) yield of various plant extracts isolated by Soxhlet's method in serial solvents.**

| S. No. | Plant Name | Plants Part Used | Percent Yield of Plant Extracts In Serial Solvents | | | | |
|---|---|---|---|---|---|---|---|
| | | | Petroleum Ether | Chloroform | Acetone | Methanol | Water |
| 1 | *D. metel* | Leaves | 0.89 | 1.29 | 2.87 | 3.10 | 3.40 |
| 2 | *C. quadrangularis* | Leaves | 0.92 | 2.01 | 2.40 | 4.01 | 4.05 |
| 3 | *P. kurroa* | Leaves | 1.04 | 2.98 | 2.97 | 3.56 | 3.80 |
| 4 | *A. catechu* | Leaves | 0.94 | 2.97 | 3.12 | 3.15 | 4.17 |
| 5 | *B. aristata* | Leaves | 0.80 | 1.90 | 3.00 | 4.77 | 5.76 |
| 6 | *T. cordifolia* | Leaves | 1.33 | 2.67 | 3.09 | 3.02 | 4.99 |
| 7 | *S. asoca* | Leaves | 1.60 | 2.87 | 3.44 | 4.88 | 3.88 |
| 8 | *E. officinalis* | Leaves | 0.90 | 2.90 | 4.00 | 4.77 | 5.77 |
| 9 | *P. pinnata* | Leaves | 1.03 | 1.90 | 3.10 | 3.23 | 3.11 |
| 10 | *C. tora* | Leaves | 1.44 | 2.54 | 5.70 | 3.66 | 3.89 |

**MIC of plant extracts using Resazurin Microtitration Dilution Assay (RMDA)**

Fifty different crude extracts of ten medicinal plants were screened for their antibacterial potential. MIC values for different extracts obtained for the pathogenic bacteria species representing antibacterial activity (Table 4). Methanol extract of *C. quadrangularis* showed promising activity where as water extract of *S. asoca* showed maximum activity against *Pseudomonas aeruginosa* (MIC 0.15 mg/mL). Antimicrobial activity of bark of *S. ascoca* was reported by Dabur et al [36]. It has been shown that antimicrobial activity of *S. asoca* bark is depends upon the catechins present in the bark [37]. Less or negligible activity was observed in all the extracts of *Tinospora cordifolia*. Aqueous and methanol extract of other nine plants normally showed antimicrobial activity in a range of 5.0 to 0.62 mg/mL. Comparatively lower activities were observed in petroleum ether and chloroform extracts (MIC 5.0 to 1.25mg/mL). Streptomycin is used in this study as positive control shows MICs in the range of 0.15-1.25 mg/mL against the bacterial strains used in the study. After the overall observation of the MIC results it is concluded that the aqueous and methanol extracts from the studied plants showed broad range of activity and could be potential source of antimicrobial compounds.

**Table 4: MIC of plant extracts against the pathogenic bacteria by Resazurin microtitre dilution assay.**

| Plant name | Solvent Fraction | MIC (mg/mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **B s** | **S a** | **S t** | **E c** | **K p** | **P a** |
| *P. kurroa* | Petroleum Ether | 5.0 | - | - | - | 5.0 | 2.5 |
| | Chloroform | - | 2.5 | - | 5.0 | 1.25 | - |
| | Acetone | 2.5 | 5.0 | 2.5 | - | - | - |
| | Methanol | 1.25 | 1.25 | - | 0.62 | 5.0 | 2.5 |
| | Water | - | - | - | 1.25 | 1.25 | 1.25 |
| *D. metel* | Petroleum Ether | 5.0 | - | - | - | 1.5 | 1.25 |
| | Chloroform | 1.25 | - | 5.0 | 5.0 | - | 2.5 |
| | Acetone | 2.5 | - | - | 5.0 | 2.5 | - |
| | Methanol | 2.5 | 5.0 | - | 1.25 | - | - |
| | Water | 1.25 | 1.25 | - | - | 5.0 | 1.25 |
| *A. catechu* | Petroleum Ether | 2.5 | - | - | - | - | 5.0 |
| | Chloroform | 2.5 | 2.5 | 2.5 | 5.0 | 3.0 | - |
| | Acetone | 5.0 | - | - | 5.0 | 3.0 | - |
| | Methanol | 1.25 | 1.25 | - | 0.62 | 2.5 | 2.5 |
| | Water | 5.0 | - | - | 2.5 | 5.0 | 2.5 |
| *C. quadrangularis* | Petroleum Ether | 5.0 | 5.0 | 5.0 | - | - | - |
| | Chloroform | - | - | 2.5 | - | - | - |
| | Acetone | - | 5.0 | 5.0 | - | 1.25 | 2.5 |
| | Methanol | 0.62 | 0.62 | 1.25 | - | - | 2.5 |
| | Water | - | 2.5 | - | - | 1.25 | - |
| *C. tora* | Petroleum Ether | 5.0 | - | - | - | 5.0 | - |
| | Chloroform | 5.0 | - | - | - | - | - |
| | Acetone | 2.5 | - | 2.5 | - | 5.0 | 2.5 |
| | Methanol | - | - | - | - | 2.5 | - |
| | Water | - | 0.62 | - | 1.25 | 5.0 | 2.5 |
| *B. aristata* | Petroleum Ether | - | - | 5.0 | - | - | - |
| | Chloroform | - | - | - | - | - | - |
| | Acetone | - | 5.0 | 5.0 | - | - | 2.5 |
| | Methanol | - | - | - | - | - | 2.5 |
| | Water | - | 2.5 | - | 5.0 | - | 5.0 |
| *P. pinnata* | Petroleum Ether | - | 5.0 | - | 2.5 | - | - |
| | Chloroform | 5.2 | 5.0 | - | 5.0 | - | 5.0 |
| | Acetone | - | 5.0 | - | 2.5 | 2.5 | 5.0 |
| | Methanol | 2.5 | 5.0 | - | 5.0 | - | 5.0 |
| | Water | - | 5.0 | 2.5 | - | - | - |
| *E. officinalis* | Petroleum Ether | - | - | - | - | - | - |
| | Chloroform | - | - | 5.0 | - | - | - |
| | Acetone | - | - | - | - | 2.5 | 5.0 |
| | Methanol | - | 5.0 | - | 5.0 | 2.5 | 5.0 |
| | Water | - | 5.0 | 2.5 | - | 5.0 | - |
| *S. asoca* | Petroleum Ether | - | - | - | - | 5.0 | 2.5 |
| | Chloroform | - | 5.0 | - | - | 5.0 | 2.5 |
| | Acetone | - | 5.0 | - | 3.4 | 3.1 | 5.0 |
| | Methanol | 5.0 | 1.25 | - | 1.25 | 1.25 | 0.62 |
| | Water | 1.25 | 2.5 | 2.5 | 2.0 | - | 0.15 |
| *T. cordifolia* | Petroleum Ether | - | - | - | - | - | - |
| | Chloroform | 5.0 | - | - | - | 5.0 | - |
| | Acetone | - | - | - | - | - | - |
| | Methanol | - | - | - | 5.0 | - | 5.0 |
| | Water | - | - | - | - | - | - |

(**B s**= *Bacillus subtilis*); (**S a**= *Staphylococcus aureus*); (**S t**= *Salmonalla typhi*); (**E c**= *Escherichia coli*); (**K p**= *Klebsiella pneumonia*); (**P a**= *Pseudomonas aeruginosa*) and (-) means no activity above 5 mg/mL.

## CONCLUSION

The results of present study reveal that the antibacterial potential of medicinal plants varies with the species of the plants and solvents used for the extraction of phytoconstituents. The active extracts serve as reservoir of potential lead compounds. Further studies on *C. quadrangularis* and *S. asoca* may lead toward discovery of novel antimicrobial compounds. As on date new molecules are being identified and comprehensive Quantitative Structural Activity Relationship (QSAR) can be carried out in order to find an optimal combination of pharmacologically important substructure groups. Further structural correlation based studies lead the drug industries to develop compounds of more efficacy. Research may also be channelled towards proteome based studies of differential expressed microbial proteins (secretary & cellular) after treatment with bioactive compounds to identify the target proteins.

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## REFERENCES

1. Gahlaut A, Pawar SD, Mandal TK, Dabur R. Biochemical analysis of lithiasis patients and treatment study using Bryophyllum pinnatum salisb. Int J Pharmacy and Pharma Sci 2012; 4: 505-507.
2. Koppula S, Ammani K, Bobbarala V, Bramhachari PV. Antibacterial activity screening of few medicinal plants from the southern region of India. Journal of Pharma Res 2010; 3: 2453-2456.
3. Clark AM. Natural Products as a resource for new drugs. Pharma Res 1996; 13: 1133-1141.
4. Jain SP, Verma DM, Singh SC, Singh JS, Kumar S. Flora of Haryana. Central Institute of Medicinal and Aromatic plants. Lucknow, India 2000.
5. Rathee D, Rathee P, Rathee S, Rathee D. Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. Arab J of Chem 2012, Article in Press.
6. Rajaprabhu D, Rajesh R, Jeyakumar R, Buddhan S, Ganesan B, Anandan R. Protective effect of Picrorhiza kurroa on antioxidant defense status in adriamycin-induced cardiomyopathy in rats. J of Med Plant Res 2007; 1: 080-085.
7. Husain GM, Singh PN, Kumar V. Antidiabetic activity of standardized extract of Picrorhiza kurroa in rat model of NIDDM. Drug Discov Ther 2009; 3: 88-92.
8. Dabur R, Diwedi SK, Yadav V, Mishra V, Singh R, Singh H, Sharma GL. Efficacy of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate in a murine model of invasive aspergillosis. Antimicrob Agen and Chemotherap 2005; 49: 4365-4367.
9. Dabur R, Chhillar AK, Yadav V, Kamal PK, Gupta J, Sharma GL. *In vitro* antifungal activity of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl) -1-methylethyl pentanoate, a dihydropyrrole derivative. J of Med Microbio 2005; 54: 549-552.
10. Rajesh, Sharma GL. Studies on antimycotic properties of Datura metel. J of Ethnopharmaco 2002; 80: 193-197.
11. Negi BS, Dave BP. *In Vitro* Antimicrobial Activity of Acacia catechu and Its Phytochemical Analysis. Indian J Microbiol 2010; 50: 369–374.
12. Ismail S, Asad M. Immunomodulatory activity of *Acacia catechu*. Indian J Physiol Pharmacol 2009; 53: 25-33.
13. Lakshmi.T, Roy A, Geetha RV. *"Acacia catechu Willd* –A gift from ayurveda to mankind" –A review. The Pharma Res 2011; 5: 273-293.
14. Nagaraja TG, Sarang SV, Jambhale DC. Evaluation of anti-mycotic activity of *Acacia catechu* Willd. (Mimosaceae). J of Biopesticides 2008; 1: 197-198.
15. Udupa KN, Prasad GC, Sen SP. The effect of phytogenic steroid in the accleration of fracture repair. Life science 1965; 4: 317.
16. Jainu M, Devi CSS. Effect of *Cissus quadrangularis* on gastric mucosal defensive factors in experimentally induced gastric ulcer-A comparative study with sucralfate. J Med Food 2004; 7: 372-376.
17. Singh LM, Udupa KN. Studies of *C. quadrangularis* in fracture by using phosphorus. Ind J Med Sci 1962; 76: 926-931.
18. Luseba D, Elgorashi EE, Ntloedibe DT, Staden JV. Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. South African J of Bot2007; 73: 378-83.
19. Rajpal V. Standardization of Botanicals. Eastern Publishers 2005; 1: 77-81.
20. Tiwari P, Kumar K, Panik R, et al. Hepatoprotective effects of *Cassia tora* whole plant. Inter Jof Pharma and Tech 2011; 3: 2798-2806.
21. Yen GC, Wu CH. Antigenotoxic properties of Cassia tea (*Cassia tora* L.): Mechanism of action and the influence of roasting process. Life Sci 2004; 76: 85-101.
22. Hyun SK, Lee H, Kang SS, Chung HY, Choi JS. Inhibitory activities of *Cassia tora* and its anthraquinone constituents on angiotensin converting enzyme. Phytother Res 2009; 23: 178-184.
23. Patel RP, Patel KC. Antibacterial activity of *Cassia tora* and *Cassia obovata*. Ind J of Pharma 1957; 19: 70-73.
24. Pal M, Mukherjee PK, Saha K, Saha BP. Antifungal activities of the leaf extract of *Cassia tora* Linn. (Fam. Leguminosae). Phytother Res 1996; 10: 521-522.
25. Sharma C, Aneja KR, Kasera R. Screening of *Berberis aristata* for antimicrobial potential against the pathogens causing ear infection. Int J of Pharmacol 2011; 7: 536-541.
26. Shahid M, Rahim T, Shahzad A, Tajuddin, Latif A, Fatma T, Rashid M, Raza A, Mustafa S. Ethnobotanical studies on *Berberis aristata* DC. root extracts. Af J of Biotechnol 2009; 8: 556-563.
27. Das S, Das MK, Mazumder PM, Das S, Basu SP. Cytotoxic Activity of Methanolic Extract of *Berberis aristata* DC on Colon Cancer. Glob J of Pharma 2009; 3: 137-140.
28. Srinivasan K, Muruganandan S, Lal J. Evaluation of antiinflammatory activity of *Pongamia pinnata* leaves in rats. J Ethnopharmacology 2001; 78: 151-157.
29. Mahli SS, Basu SP, Sinha KP, Banarjee NC. Pharmacological effects of Karanjin and Pongamol. Ind J of Animal Sc 1989; 59: 657-660.
30. Chandrashekar KS, Prasanna KS. Antimicrobial activity of *Pongamia pinnata* leaves. Int J Med Res 2010; 1: 18-20.
31. Javale P, Sabnis S. Antimicrobial properties and phytochemical analysis of *Emblica officinalis*. Asian J Exp Biol Sci 2010: 91-95.
32. Prathapana A, Lijo Cheriana, O, Nampoothiria SV, Minib S, Raghu KG. *In vitro* antiperoxidative, free radical scavenging and xanthine oxidase inhibitory potentials of ethyl acetate fraction of *Saraca ashoka* flowers. Nat Product Res 2011; 25: 298–309.
33. Seetharam N, Sujeeth H, Jyothishwaran G, Barad A, Sharanabasappa G, Shabana P. Antibacterial activity of *Saraca asoka* bark. Ind J P Sci 2003; 4: 658-659.
34. Varghese CD, Satish NC, Pannikar KR. Potential anticancer activity of *Saraca asoca* extracts towards transplantable tumours in mice. Ind J P Sci 1992; 54: 37-40.
35. Gahlaut A, Taneja P, Shirolkar A, Nale A, Hooda V, Dabur R. Principal component and partial least square discriminant based analysis of methanol extracts of bark and re-generated bark of *Saraca Asoca*. Int J of Pharma and Pharmaceut Sci 2012; 4: 331-335.
36. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS. Antimicrobial activity of some medicinal plants. Af J of Trad, Complement and Alt Med 2007; 4: 313-318.
37. Shirolkar A, Gahlaut A, Chhillar A, Dabur R. Quantitative analysis of catechins in *Saraca asoca* and correlation with antimicrobial activity. J of Pharma Anal 2013. http://dx.doi.org/10.1016/j.jpha.2013.01.007.
38. Kedar NP, William CC, Bipin K. Multiple antioxidants in prevention and treatment of Parkinson's disease. J of Am Coll of Nutr 1999; 18: 413-23.
39. Gahlaut A, Gothwal A, Dabur R. TLC based analysis of allelopathic effects on tinosporoside contents in Tinospora cordifolia. J of Chem and Pharma Res 2012; 4: 3082-3088.

40. Shirolkar A, Gahlaut A, HoodaV, Dabur R. Phytochemical composition changes in untreated stem juice of Tinospora cordifolia (W) Mier during refrigerated storage. J of Pharma Res 2013. http://dx.doi.org/10.1016/j.jopr.2013.01.018.
41. Sarkar DS, Lutfun N, Kumara Y. Microtitre plate-based antibacterial assay incorporating rezazurin as an indicator of cell growth and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 2007; 42: 321-4.
42. McNicholl BP, McGrath JW, Quinn JP. Development and application of a resazurin-based biomass activity test for activated sludge plant management. Water Res. 2006; 41: 127–133.
43. Bigalke DL. Methods used for monitoring the microbiological quality of raw milk. Dairy Food Sanit 1984; 4: 189–190.