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COMPARATIVE ANTIMICROBIAL ACTIVITY OF DIFFERENT SPICES OF *CASSIA* L. IN SELECTIVE MICROORGANISM.

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ABSTRACT

The potential of higher plants as source for new drugs is still largely unexplored. Medicinal plants represent a rich source of antimicrobial agents. In present investigation, there were three different plants species of *Cassia* L. namely *Cassia fistula* L., *C. tora* L. and *C. occidentalis* L. were taken on the account for investigation on antimicrobial activity of aqueous extracts of their leaves against some common pathogenic gram +ve and gram –ve pathogenic bacterials strains. The leaves of three plant species namely *Cassia fistula* L., *C. tora* L. and *C. occidentalis* L. were collected and subjected to soxhlet extraction with distilled water to prepare aqueous extracts. 4 dilutions of 100 mg/ml stock of extract was used to perform antimicrobial activity using Kirby-Bauer method. All the extracts were reported to show their antimicrobial acitivtiy against the test Gram +ve & Gram –ve bacteria including *Pseudomonas aeruginosa* (MTCC-*1934), *Shigella flexneri* (MTCC-1457), *Escherichia coli* (MTCC-1687) *Staphylococcus aureus* (MTCC-737), *Staphylococcus epidermidis* (*MTCC*-3615) and *Bacillus subtilis* (MTCC-7164). The results of present investigation encourages the development of new therapeutic and antimicrobial drug of plant origin upon further extensive investigations.

K**eywords:** Cassia, antimicrobial activity, Phytodrug, natural therapeutics

INTRODUCTION

The potential of medicinal plants is boundless in terms of renewable chemical and biological resourcing in terms of developing new pharmaceuticals[1,2,3]. Since India is astonishingly abundant in its flora, with more than lakhs of herbal species with potential medicinal importance typically due to their low side-effects, low cost and a high level of acceptance by and majority of the population including people with pathological conditions[4,5,6].

The important bioactive compounds of plants are counted as alkaloids, flavonoids, tannins and phenolic compounds. It would be an effective approach to conduct ethno-pharmacological information based research on phytochemical research in the discovery of new anti-infective agents from higher plants[7].

The potential of higher plants as source for new drugs is still largely unexplored. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[8]. With reference to the web resources genus *Cassia* forms

the major group of *Angiosperms* (Flowering plants) in the family *Leguminosae*. (The Plant List) (http://www.theplantlist.org/browse/A/Leguminosae/Cassia/). There are several plant species that comes under genus *Cassia* L. and several work have been conducted on various aspects of the plants of this genus. But in present investigation, there were three different plants species of *Cassia* L. namely *Cassia fistula* L., *C. tora* L. and *C. occidentalis* L. were taken on the account for investigation on antimicrobial activity of aqueous extracts of their leaves against some common pathogenic gram +ve and gram –ve pathogenic bacterials strains which may result into the development of potent natural remedy for many infections after advance studies in future.

MATERIALS AND METHODS

Collection of plant material

The leaves of three plant species of Cassia namely *Cassia fistula* L., *C. tora* L. and *C. occidentalis* L. were collected from the well identified plants from the road sides within the Bhopal city. The identification of the complete plant and its parts were done by Dr. Madhuri Modak, Professor at Department of Botany, Government College, Ashta, Madhya Pradesh, India.

Collection of Pathogenic microbes

The test pathogenic strain Gram +ve & Gram –ve bacteria including *Pseudomonas aeruginosa* (MTCC-*1934), *Shigella flexneri* (MTCC-1457), *Escherichia coli* (MTCC-1687) *Staphylococcus aureus* (MTCC-737), *Staphylococcus epidermidis* (*MTCC*-3615) and *Bacillus subtilis* (MTCC-7164) were resourced from IMTech Chandigarh.

Extraction of phytochemicals

The leaves of *C. fistula* L., *C. tora* L. and *C. occidentalis* L. were washed, cleaned and dried at room temperature for 2-3 days then grounded into fine powder using electric grinder. After defatting in petroleum ether overnight the aqueous extracts of all the three plant materials were prepared separately by soxhlation method which is then concentrated by evaporating solvent (H_2O) in water bath[9, 10]. The extract so obtained was used for their antimicrobial activity.

Antibacterial activity of extracts

Each lyophilized MTCC microbial cultures were first revived in nutrient broth separately. The stock solution of extracted phytochemical mixture from each plant was prepared in 100 mg/ml by dissolving extracts sterile distilled water followed by preparation of serial dilution of antimicrobial activity. The disc diffusion method was used to determine the antimicrobial activity of the aqueous extracts prepared from the leaves of *C. fistula* L., *C. tora* L., and *C. occidentalis* L. using standard Kirby-Bauer method[11].

Using sterile cotton swab the broth inoculum of test organism was applied on to the surface of nutrient agar plates following aseptic techniques in laminar air flow hood work bench (NU-157, Navyug India). After 5-10 minutes, the pre-soaked & dried Whatman filter paper discs (no.1) of 6 mm diameter in different concentrations of phytochemical extracts of plants were placed on to the surface of pre-inoculated agar media plates[12]. The plates were then incubated at 37^{o}C for 24 hours to see any clear zones of inhibition around the discs. This experiment was repeated with each test microorganism for different plant extracts.

RESULTS & DISCUSSION

The results of antimicrobial activity of aqueous extracts of the three different plant species of Genus Cassia L. against the Gram +ve & Gram –ve bacteria are depicted in table 1, table 2 and table 3. From the experimental outcomes it is indicated that the aqueous extract of leaved of the three plants namely *C. fistula* L., *C. tora* L., and *C. occidentalis* L. are efficiently inhibiting test microbial species at 0% dilution of stock with 100 mg/ml extract concentration using disc diffusion technique. The inhibitory potential of the extracts declines with decrease in extract concentration in case of all the extracts against the test bacterial species and it is could be considered significant inhibition upto 50% dilution of extract stocks.

The maximum zone of inhibition against *S. flexneri*, at 0% dilution i.e. 100% concentration, *C. fistulosa* extract produced 14 mm zone, *Cassia tora* extract produced 12 mm zone and *C. occidentalis* extract gave 10 mm zone size. Whereas the minimum zone of inhibition at 100% concentration was observed to be 9 mm against *S. aureous* due to *C. fistula* L., 08 mm due to *C. tora* L., against *P. aeruginosa* and 07 mm due to *C. occidentalis* L. against *S. aureous* was observed. The variation in inhibitory potential of aqueous extracts of the three different plants species of same genus are described comparatively throurh graphs depicted in figure 1a & 1b, figure 2a & 2b, and figure 3a & 3b.

Table 1: Results of antibacterial activity on test gram +ve and gram –ve bacteria due to *C. fistulosa* extract

S.N	Bacterial Strains	Used Bacteria	Zone of inhibition (in mm) at different extract concentration			
			100%	***75%***	***50%***	***25%***
1.	Gram –ve bacteria	*P. aeruginosa*	10	09	09	08
		S. flexneri	14	12	09	05
		E. coli	11	10	08	07
2.	Gram +ve bacteria	*S. epidermidis*	11	09	08	06
		S. aureous	09	07	08	07
		B. subtilis	12	10	08	06

Table 2: Results of antibacterial activity due *Cassia tora* leaf aqueous extract against gram +ve and gram –ve bacteria

S.N	Bacterial Strains	Used Bacteria	Zone of inhibition (in mm) at different extract concentration			
			100%	***75%***	***50%***	***25%***
1.	Gram –ve bacteria	*P. aeruginosa*	08	07	07	06
		S. flexneri	12	09	08	07
		E. coli	10	08	07	06
2.	Gram +ve bacteria	*S. epidermidis*	09	08	06	05
		S. aureous	10	07	09	06
		B. subtilis	09	07	06	06

Table 3: Results of antibacterial activity on test gram +ve and gram –ve bacteria due *C. occidentalis* extract

S.N	Bacterial Strains	Used Bacteria	Zone of inhibition (in mm) at different extract concentration			
			100%	***75%***	***50%***	***25%***
1.	Gram –ve bacteria	*P. aeruginosa*	08	08	06	05
		S. flexneri	10	09	07	06
		E. coli	09	08	08	05
2.	Gram +ve bacteria	*S. epidermidis*	08	09	07	06
		S. aureous	07	06	06	05
		B. subtilis	10	08	07	06

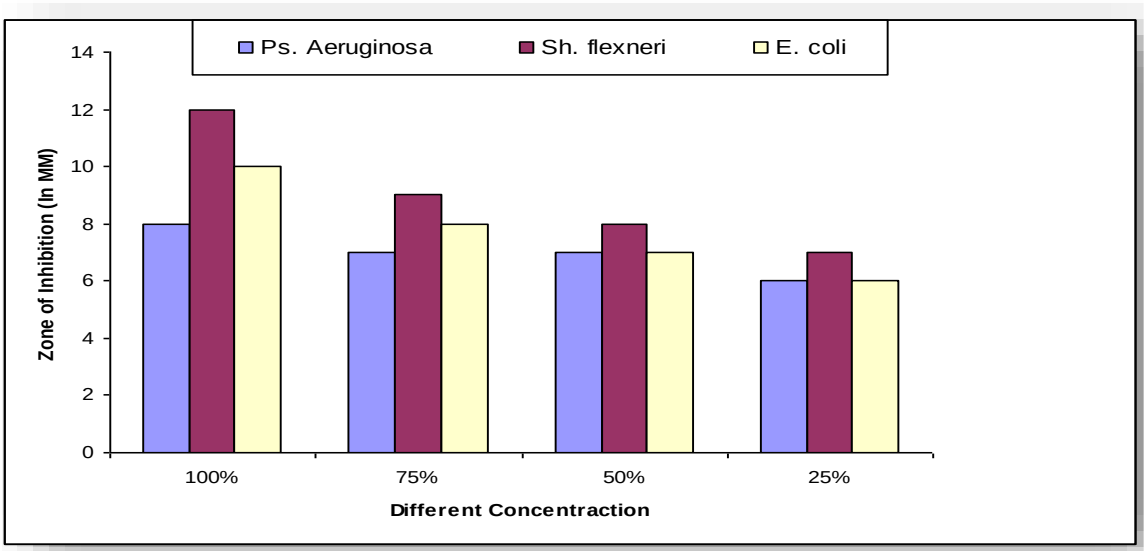

Figure 1a: Graph showing antibacterial activities of extract of aqueous Cassia *tora* against Gram Negative bacterial stain

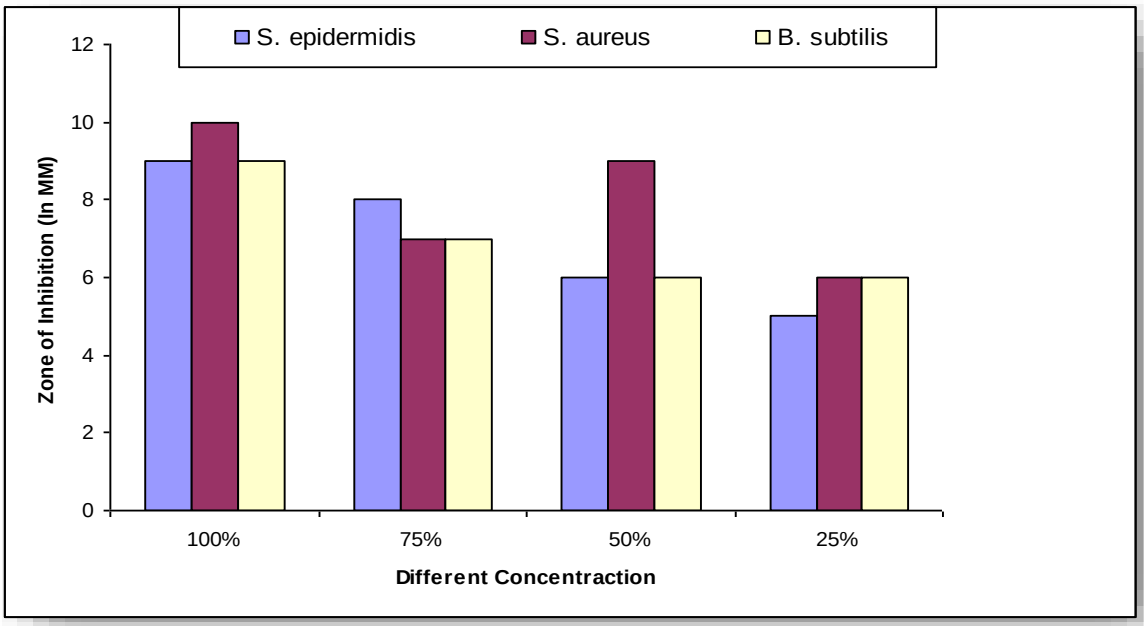

Figure 1b: Graph showing antibacterial activities of extract of aqueous *Cassia tora* against Gram Positive bacterial stain

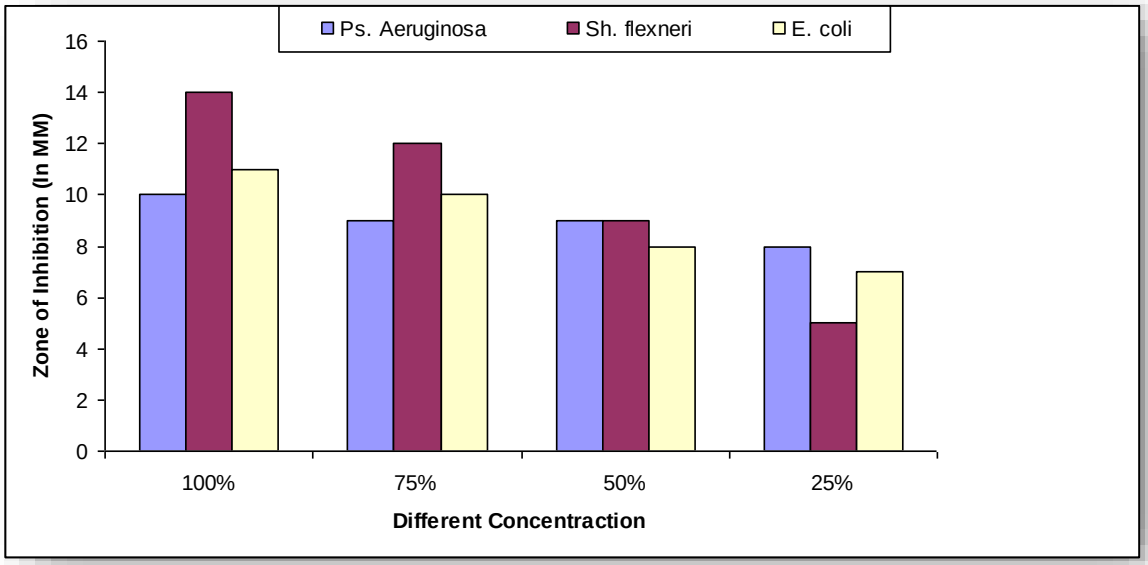

Figure 2a: Graph showing antibacterial activities of aqueous extract of *C.fistula* against gram negative bacterial stain

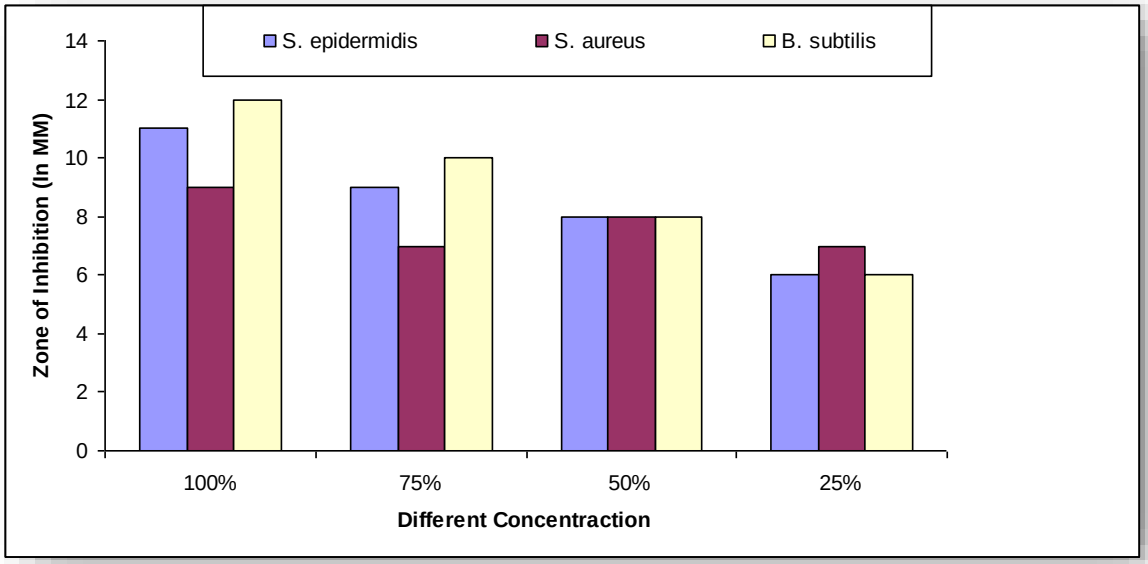

Figure 2b: Graph showing antibacterial activities of aqueous extract of *C.fistula* against gram positive bacterial stain

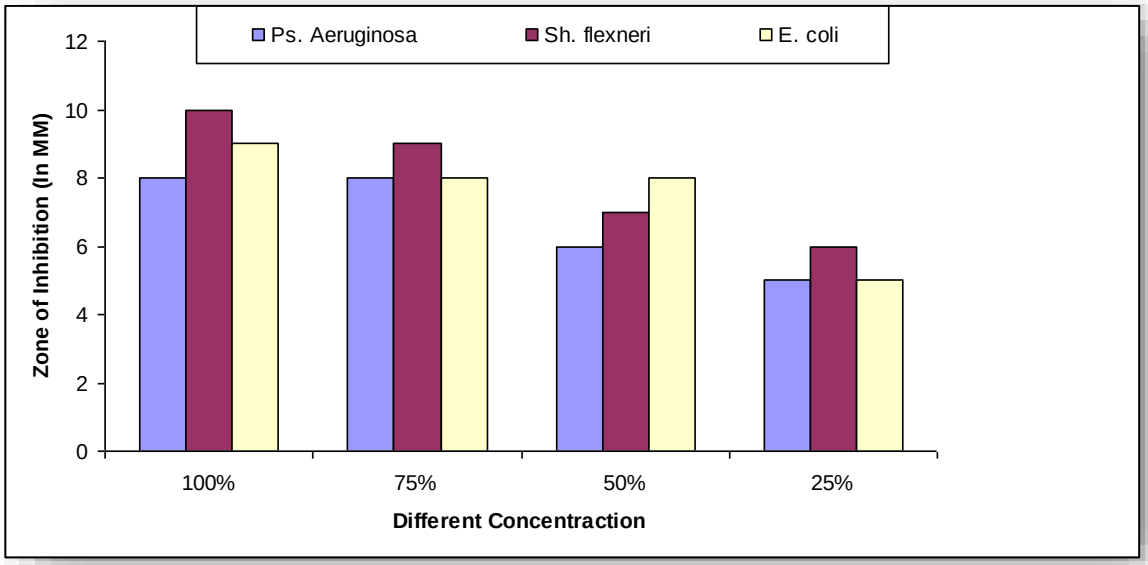

Figure 3a: Graph showing antibacterial activities of aqueous extract of *Cassia occidentalis* against Gram Negative bacterial stain

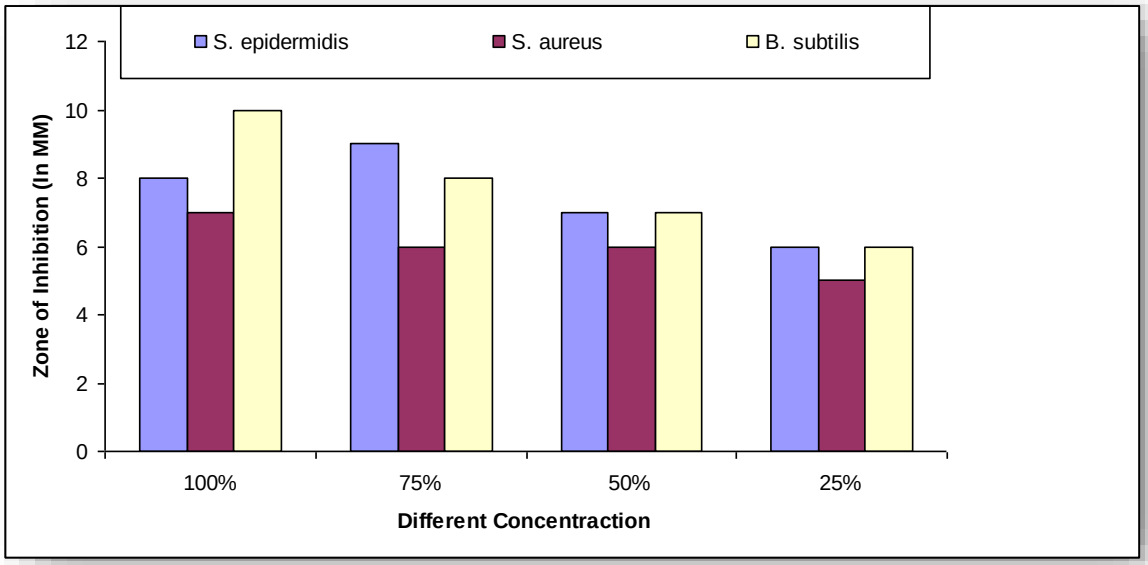

Figure 3b: Graph showing antibacterial activities of aqueous extract of *Cassia occidentalis* against Gram Positive bacterial stain

With reference to literature water soluble flavonoids & phenolics does not pose any antimicrobial significance, but the antioxidant potential[13]. Many health issues and illness are associated with infections due to common pathogens, sometimes leads serious health concern even death[14]. Because of the side effects of routine antimicrobial drugs, medical practioners & scientist are working on alternative natural antimicrobial drugs[15]. Plant polyphenols studied with a thought that they might be imposing protective effects afforded by fruit and vegetable intake against many chronic diseases including cancer. The quantity of phenolic substance in any extract of plant correspond to the antimicrobial, antioxidant potential and other pharmacological effects[10].

Rajni, *et al*., (2014)[16] evaluated the response of respiratory tract pathogens *S. aureus S. pneumoniae* and *S. pyogenes* towards the petroleum ether, acetone, methanol, and aqueous extracts of seed of *C. occidentalis* L. Panda *et al.,* (2011)[17] also worked out the antimicrobial activity of *C. fistua* L., against *E.coli*, *Salmonella typhimurium*, *Shigella sonnei*, *B.subtilis*, *B. licheniformis* , *S. aureus* and *S. epidermidis* using multiple number of extracts including petroleum ether, chloroform, methanolic, ethanol, and aqueous extracts of leaves. Bhalodia, and Shukla (2011) also confirmed the antibacterial and antifungal potentials of hydroalcoholic extracts of *C. fistula* L. leaves[18]. While, Chavan, *et al*., (2011) reported that the ethanolic and aqueous extracts from the leaves of *Cassia tora* at concentrations 0.15mg, 0.31 mg respectively exhibiting significant antibacterial and antifungal activity using ATCC culture of *E. coli, P. aeruginosa, S. aureus, B. subtillus, A. niger* and *C. albicans* compared with standard ciprofloxacin[19].

Present investigation also confirmed that the phytochemical extracts from the three plants under investigation were sufficiently inhibiting the test pathogenic bacterial species including Gram –ve *Pseudomonas aeruginosa*, *Shigella flexneri* and *Escherichia coli* bacterium and Gram +ve *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*.

Conclusions

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[20]. The aqueous extract of leaves of Cassia namely *Cassia fistula* L., *C. tora* L. and *C. occidentalis* L. were rich in various types of phytochemicals because of which imparted antimicrobial potential towards the test microbial species encourages the prospects of these plant extract in medicinal and therapeutic significance due to the presence of bioactive phytoconstituents that could lead to the development of cost effective healing alternatives in future upon further extensive investigation related to its safe use for humans and animals.

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References

1. Fransworth NR. Preclinical assessment of medicinal plants. Natural Resources and Human Health. Elsevier Science Publishers BV. 1992; 87-91.
2. Padulosi S, Leaman D and Quek P. Challenges and opportunities in enhancing the conservation and use of medicinal and aromatic plants. J Herbs Spices Med Plants. 2002; 9: 243-267
3. Mamedov N. Medicinal Plants Studies: History, Challenges and Prospective. Medicinal & Aromatic Plants. 2012; 1:8.
4. Wilt TJ, Ishani A, Rutks I, and MacDonald R. Phytotherapy for benign prostatic hyperplasia. Public Health Nutrition. 2000; 3:459–472.
5. Vanderhoof JA. Probiotics: future directions. Amnals of Journal of Clinical Nutrition. 2001; 73:1152S-1155S.
6. Rajapandian G, Narayanan N, Maheswaran A, and Chanchal P. Dangerous world of counterfeit drugs–Pharmacist's role and its prevention. Int J Pharm Sci Rev Res. 2013; 4: 1709–1713.
7. Duraipandiyan V, Ayyanar M and Ignacimuthu S. Antimicrobial Activity of Some Ethnomedical Plants Used by Paliyar Tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006; 635.
8. Srivastava J, Lambert J and Vietmeyer N. Medicinal Plants: An Expending Role in Development. World Bank Technical Paper. 1996; 320.
9. Handa SS. Extraction Technologies of Medicinal and Aromatic Plants. ICS-UNIDO, International Centre for Science and High Technology. Trieste. 2008; 21-33.
10. Tenguria M, Chand P and Upadhyay R. Estimation of Total Polyphenolic Content in Aqueous and Methanolic Extracts from the Bark of Acacia nilotica. Int J Pharm Sci Res. 2012; 3(9): 3458-3461.
11. Bauer AW, Kirby WM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4):493-6.
12. Ingraham JL and Ingraham CA. Microbiology an Introduction. Cengage Learning, Indian Edition. 2005.
13. Das K, Tiwari RKS and Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research. 2010; 4(2): 104-111.
14. Park K and Park. Textbook of Preventive and Social Medicine. Sanghavi Printing Press, Jabalpur, 1994.

15. Zgoda JR and Porter JR. A Convenient microdilution method for screening of natural products against bacteria and fungi. Pharm. Biol. 2001; 39(3): 221-225.
16. Rajni, Gautam SS and Navneet. Antibacterial and phytochemical analysis of Cassia occidentalis L. seeds against respiratory tract pathogens. Indian Journal of Natural Products and Resources. 2014; 5(1): 52-55.
17. Panda S, Padhi LP and Mohanty G. Antibacterial activities and phytochemical analysis of Cassia fistula (Linn.) leaf. Journal of Advance Pharmaceutical Technolology and Research. 2011; 2(1): 62–67.
18. Bhalodia NR and Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. Journal of Advanced Pharmaceutical Technology and Research. 2011; 2(2): 104–109.
19. Chavan RT, Deshmukh VL and Kadam AS. Pharmacology Antibacterial Activity of Cassia tora Leaves. Recent Research in Science and Technology. 2011; 3(5): 12-14.
20. Tona L, Kambu K, Ngimbi N, Cimanga K and Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology. 1998; 61: 57-65.