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Evaluation of Antibacterial Potency and Phytochemical Screening of Seed Extracts of *Butea monosperma* (LAM.).

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ABSTRACT

The success of treatment against infectious diseases was achieved through the constant search of novel drugs to manage the problems caused by resistant microbial strains. Still infectious agents developing resistance to the existing synthetic drugs now becoming major problem, so it is important to identify novel sources of antimicrobial agents as an alternative. The sequential extraction of *Butea monosperma* seed extracts displayed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, saponins, sterols and tannins. The antimicrobial activity of seed extracts were analyzed using *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* using agar well diffusion method. Maximum activity was noticed in methanol fraction and it had the highest inhibition zone - size (16 mm) against *S. aureus.* Ciprofloxacin 5 µg/disc was used as reference control and 10% dimethyl sulfoxide as negative control. The minimum inhibitory concentration (MIC) of methanol extract against bacterial strains was 125 µg/ml and that of aqueous extract was 250 µg/ml. The FTIR spectrum generated characteristic peaks in the region from 400 – 4000 cm^{-1}, indicated the presence of various functional groups of prepared extract. So it is evident that there is more possibility of developing new antimicrobial drugs using this plant which leads to the development of new pharmaceuticals.

Keywords: Antimicrobial susceptibility test, *Butea monosperma*, Minimum inhibitory concentration, phytochemical constituent.

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INTRODUCTION

The use of antibiotic agents is considered one of the most powerful tools to prevent and treat bacterial infections. Although, in past three decades the indiscriminative use of antibiotics often leads to dramatic increase in microbial resistance and according to World Health Organization (WHO) it is one of the biggest threats to global health, food security and development today. It is due to the emergence of new resistance mechanisms which is spreading globally, threatening our capability to treat common infectious diseases, resulting in prolonged illness, disability and death [1]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [2] and leads to the development of new multi-drug resistant bacterial strains resulting in high mortality. Due to the multi-drug resistance, the development of new synthetic antimicrobial drugs has slowed down, which leads to the search of new antimicrobials from alternate sources. The problem of microbial resistance is raising but the drug used for antimicrobial treatment in future is still unclear. Therefore, it is necessary to find other alternatives that can be effective in the treatment of these problematic microbial infections.

Since ancient time, plants and their extracts are used as promising remedies as therapeutic and agents against several infectious diseases in Ayurveda, Chinese, Arabic and Unani medicine [3]. The WHO estimated that about 80% of population from developing countries depends on phyto-drugs for their primary health care needs [4, 5]. One of the ways to prevent antibiotic resistance is to identify new compounds which are not based on the existing synthetic chemicals [6]. Plant extracts with potent phytochemicals showing antimicrobial activities can be exploited to satisfy this need, because the structures of these chemicals are vary from those that are isolated from microbial sources and so the function may also differ [7]. It is important to explore the relationship between the active principles of medicinal plants with its pharmacological activity. Novel drugs are discovered through screening of active compounds of plants which are effective in treating various diseases including cancer [8] and Alzheimer's disease [9].

Butea monosperma Lam. (Fabaceae) is an Indian medicinal tree species also known as 'Flame of the Forest'. In traditional medicine it is recommended as a health tonic. Its flowers are effective against liver disorders and reported to possess anti-implantation activity [10]. Leaves of *B. monosperma* are traditionally used as anti-inflammatory, anti-tumor, anti-microbial, anti-helminthic and stomach disorders [11]. Roots of *B. monosperma* are stated to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumors. An Indian Ayurvedic drug Pippalirasayana is prepared from *B. monosperma* and is used in the treatment of giardiasis [12]. The bark is reported to possess anti-fungal and anti-diabetic activity. Gum is useful as astringent, depurative and useful in diarrhoea, hemorrhoids, leprosy and skin diseases. The present paper deals with the crude extraction of *B. monosperma* seeds using three solvents viz., petroleum ether, methanol and water (non - polar to polar, extracted by hot extractions). These extracts were used to assess the antimicrobial property of the seeds against three microbial strains.

MATERIALS AND METHODS

Plant material and preparation of seed extract

B. monosperma seeds were obtained from medicinal plants garden, Siddha Central Research Institute, Mettur, India. The plant is authenticated by a botanist Dr. Sasikala Ethirajalu in the same institute. The seeds of the plants were washed, shade dried and ground to fine powder using electric blender. The powdered material (15 g) was defatted with petroleum ether (60 - 80°C) and then extracted successively using solvents (150 ml) of increasing polarity; methanol (>95 %) and distilled water by Soxhlet apparatus for 8h at a temperature within the boiling point of the solvent. Using whatman No. 1 filter paper the extracts were filtered while it is hot. Concentration of the filtered extracts were done under reduced pressure using rotary evaporator at 40°C and dried in desiccator. 10% dimethylsulphoxide (DMSO) was used to dissolve the resultant solid mass (30 mg) with Tween - 80 and stored in air - tight containers at -20°C for further use.

Qualitative test for phytochemicals

Screening and identification of bio active compounds in the seed extracts were carried out using standard procedures as described [13-15]. Alkaloids, carbohydrates, flavonoids, glycosides, protein, saponin, starch, sterols and tannins were assessed.

Test microbial strains and medium used

The extracts of *B. monosperma* were tested against 2 gram negative: *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 15442) and 1 gram positive: *S. aureus* (ATCC 6538) species using agar well diffusion method. The pathogenic strains used were obtained from American Type Culture Collection, Manassas, USA. All the test cultures were maintained on Muller Hinton Agar (MHA) (Hi-media, India) plates at 4°C with a sub culture period of one week. The concentration of the inoculum was maintained at 1×10^8 - 10^9 colony forming units (c. f. u)/ml by comparing with a 0.5 McFarland standard solution.

Antimicrobial susceptibility test

Agar well diffusion method [16] was used to determine the antimicrobial activity of the seed extracts. The test organisms were prepared according to the 0.5 McFarland standards to get a final concentration of 1×10^8 c. f. u/ml. MHA plates were seeded with the prepared inoculum using sterile cotton swabs and the surface of the medium was allowed to dry for about 5 min. Then the hole with a diameter of 6 mm was punched aseptically using sterile cork borer and a volume of 50 µl of different test extracts (12.5 mg/ml) were placed on to each well. Ciprofloxacin 5 µg/disc was taken as reference standard and 10% DMSO as negative control. The extracts were then allowed to diffuse in the well and the plates were wrapped with para film to avoid evaporation loss. The plates were then incubated at 37°C for 24 hrs. The experiments were performed in triplicates. Antimicrobial activity was indicated by the presence of clear inhibition zones around the wells.

Determination of Minimum inhibitory concentration (MIC)

The MIC was determined by broth dilution method [17]. Inoculum of each pathogen was prepared by suspending morphologically similar colonies in Muller Hinton broth and incubated at 37°C until the visible turbidity matches with the 0.5 McFarland standards. This will yield a suspension containing 10^8 cells/ml. The suspension was diluted in sterile water or saline to get 10^5 cells/ml before inoculation. For broth dilution assay 100 µl of the prepared inoculum was added to each test tube at a final concentration of extracts 0 – 1000 µg/ml and incubated at 37°C. The MIC was considered that the lowest concentration of the tube which did not display any visible growth. All the assays were performed in triplicates.

Fourier transform infrared spectroscopy (FTIR) analysis

The types of chemical bonds or functional groups present in compounds are identified through the potent tool, FTIR. Different seed extracts dissolved in DMSO were used for FTIR analysis. The liquid sample of each plant extract was loaded in FTIR Spectroscope (PerkinElmer Spectrum Two, Waltham, MA, USA), with a wave range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} [18].

RESULTS

Phytochemical analysis

From the phytochemical analysis, alkaloids, proteins and saponins were found to be present in all the three extracts. Carbohydrates and glycosides were present in extracts obtained with methanol and water. Starch and sterols were only found in water and methanol extracts respectively. Aqueous extract had the maximum number of bio active compounds whereas petroleum ether extract had the least number of compounds (Table 1).

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Table 1: Results of qualitative phytochemical analysis of *B. monosperma* seed extracts

Bioactive compounds	PE	MeOH	AqE
Alkaloids	+	+	+
Carbohydrates	-	+	+
Flavonoids	-	+	-
Glycosides	-	+	+
Proteins	+	+	+
Saponins	+	+	+
Starch	-	-	+
Sterols	-	+	-
Tannins	-	-	+

PE: petroleum ether; MeOH: methanol; AqE: Aqueous extract. +: presence; -: absence of phyto constituent.

Antimicrobial activity

The present study investigated the antibacterial properties of all the three extracts using agar well diffusion method. This method of testing antimicrobial activity is a common standard method recommended by the Clinical and Laboratories Standards Institute. The antibacterial activity of different seed extracts against three standard bacterial species is shown in table 2. Ciprofloxacin 5 µg per disc was used as positive control with inhibition zones (*E. coli* 19 mm*, P. aeruginosa* 18 mm and *S. aureus* 19 mm). The zones of inhibition against the three strains of different fractions of *B. monosperma* were ranged from 9 – 16 mm. From the study both alcoholic and aqueous seed extracts of *B. monosperma* exhibited antimicrobial activities against all the three pathogens tested. As seen from table 2, when the two crude extracts were compared with each other, methanol fraction showed the strongest action against *E. coli* (14 mm), *P. aeruginosa* (15 mm), *S. aureus* (16 mm) at a concentration of 12.5 mg/ml. From this report, *S. aureus*, a Gram positive bacterium showed maximum sensitivity to the methanol extract of *B. monosperma* whereas the petroleum ether extract did not indicate significant activity against the pathogens tested.

Minimum inhibitory concentration

MIC values of both the extracts, by broth dilution method against all bacterial strains are also shown in Table 2. The minimum inhibitory concentrations of different extracts ranged between 125 and 500 µg/ml. With *E. coli* the minimum MIC was noticed in methanol extract as 250 µg/ml, whereas the petroleum ether and aqueous extracts showed maximum MIC of 500 µg/ml. With *S. aureus* the minimum MIC was with methanol extract as 125 µg/ml, and the maximum MIC value was with petroleum ether and water extracts as 250 µg/ml. With *P. aeruginosa* the lowest MIC value was noticed in methanol extract as 250 µg/ml, the highest value was with petroleum ether and aqueous extracts as 500 µg/ml.

Fourier transform infrared spectrophotometer (FTIR) analysis

Based on the peak values in the region of infrared radiation, the functional group of active compounds are identified. In the current investigation involving *B. monosperma*, the results of FTIR confirmed the presence of alcohol, phenol and amine group of compounds (fig. 1-3). The FT-IR peak values and the corresponding functional groups are listed in table 3.

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Table 2: Antimicrobial activity of *B. monosperma* seed extracts

Bacteria	Petroleum ether extract		Methanol extract		Water extract		References Ciprofloxacin		Neg. control
	Zone of inhibition (mm) 12.5 mg/ml	MIC µg/ml	Zone of inhibition (mm) 12.5 mg/ml	MIC µg/ml	Zone of inhibition (mm) 12.5 mg/ml	MIC µg/ml	Ref. control Cip 5µg	MIC µg/ml	10% DMSO
Escherichia coli (ATCC 25922)	9	500	14	250	13	500	19	0.05	-
Pseudomonas aeruginosa (ATCC 15442)	9	500	15	250	12	500	18	0.25	-
Staphylococcus aureus (ATCC 6538)	11	250	16	125	13.5	250	19	0.5	-

Values are the means of three replications, MIC: Minimum Inhibitory Concentration (µg/ml), Cip: Ciprofloxacin, -: No zone of inhibition.

Table 3: FT-IR peak values of *B. monosperma* seed extracts

Name of the extracts	Peak values cm^{-1}	Bond	Functional groups
Petroleum ether	3277	OH stretching	Alcohol and phenol
	1616	C=C group	Alkanes
	1394.88	C-H stretching	Alkanes
	1011	C-N stretching	Aliphatic amines
	1075	C-N stretching	Aliphatic amines
Methanol	3278.11	O-H stretch	Alcohols
	1638.60	C-C stretch	Aromatics
	1406	N-O stretch	Nitro compounds
	1011	C-N stretching	Aliphatic amines
	1069	C-N stretching	Aliphatic amines
Water	3348	N-H stretch	Primary and secondary amines
	1011	C-N stretching	Aliphatic amines
	950.74	O-H bend	Aromatics
	595.26	Unknown	Unknown
	410.55	Unknown	Unknown

DISCUSSION

New chemotherapeutic agents are developed from medicinal plants since they produce an unlimited amount of aromatic substances, like phenols or their oxygen-substituted derivatives. *Butea monosperma* plant is well-known for its medicinal value due to the presence of several flavonoids like butein, butin and iso butyrin which are predominantly present in flowers and seeds are stated to possess anti-ovulatory and anti-implantation activities [19]. It is reported that the seed oil of *B. monosperma* has significant antibacterial and antifungal activities through in-vitro testing [20]. The seed powder of the plant is well known for its use against intestinal worms [21].

In the present investigation, methanol and water extracts of the seed showed maximum yield of phytochemical constituents. Active ingredients like saponins, tannins and phenolic compounds are responsible for the antibacterial activity of the crude extracts observed. It is evidenced by the work carried out by Metha and Bokadia (1983) on in-vitro testing of the seed oil of *B. mosnosperma*, which showed significant bactericidal and fungicidal activity. The activities of the secondary metabolites present in crude

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drugs have been correlated against the disease producing microorganisms [22]. This may be the first preliminary report on antibacterial studies of the *B. mosnosperma* seeds. The results of the study clearly indicate that both the methanol and aqueous extracts of seed showed significant antibacterial activity. It is inferred that the gram-positive strains are better inhibited by the seed extracts than the gram negative bacteria since plant extracts are more effective towards gram positive bacteria than the gram negative [23]. The minimum MIC of all the extracts were lower against *S. aureus* when compared with *E.coli* and *P. aeruginosa*. This suggests that the gram positive bacteria *S. aureus* are more susceptible to the action of seed extracts of *B. monosperma*. These results are similar to the finding of previously reported study by Shailendra S. Gaurav et al. (2008) [24]. A number of researchers have been reported the antibacterial properties of different plant extracts. Arvind R Suthar., *et al.* demonstrated the *In-vitro* studies on antibacterial properties of *B. monosperma* flower extracts by agar well diffusion method against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa.* The results indicated that the flower extract exhibited good activity against the pathogens tested [25]. Pooja Gupta., *et al*. evaluated the antibacterial activity of different plant parts of *B. monosperma* such as leaf, bark and flowers by agar well diffusion method. The results specified that alcoholic extracts of all the plant parts showed good antibacterial activities against various gram positive and gram negative strains [26]. The results obtained were reliable with the earlier works carried out by many researchers. The antibacterial property may be due to the presence of active compounds like flavonoids, saponins and phenolic compounds present in the seed extracts.

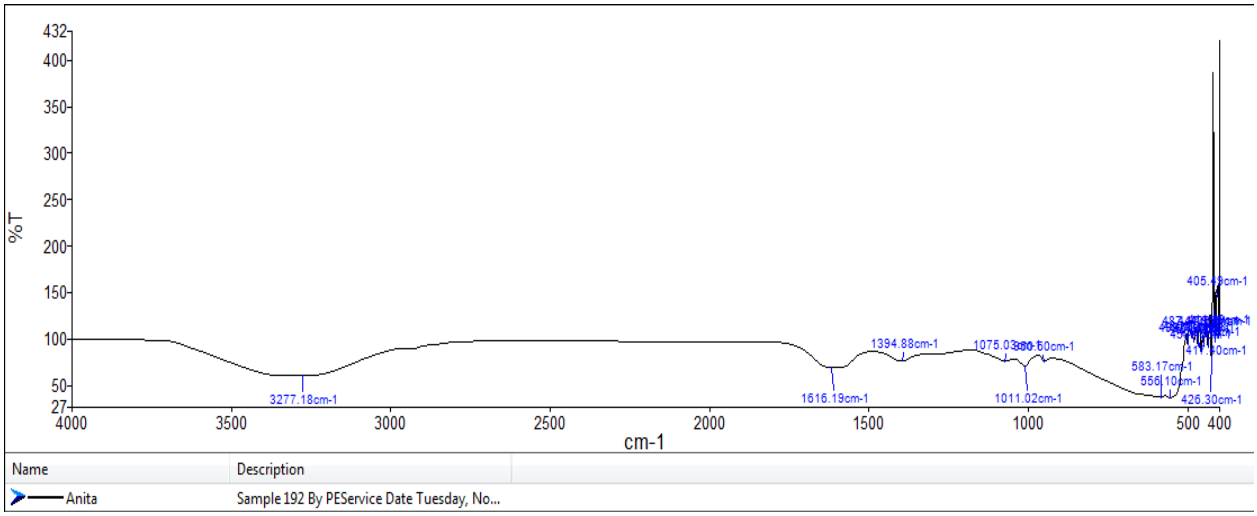

Fig 1: FT-IR spectrum of petroleum ether extract of *B. monosperma* seeds

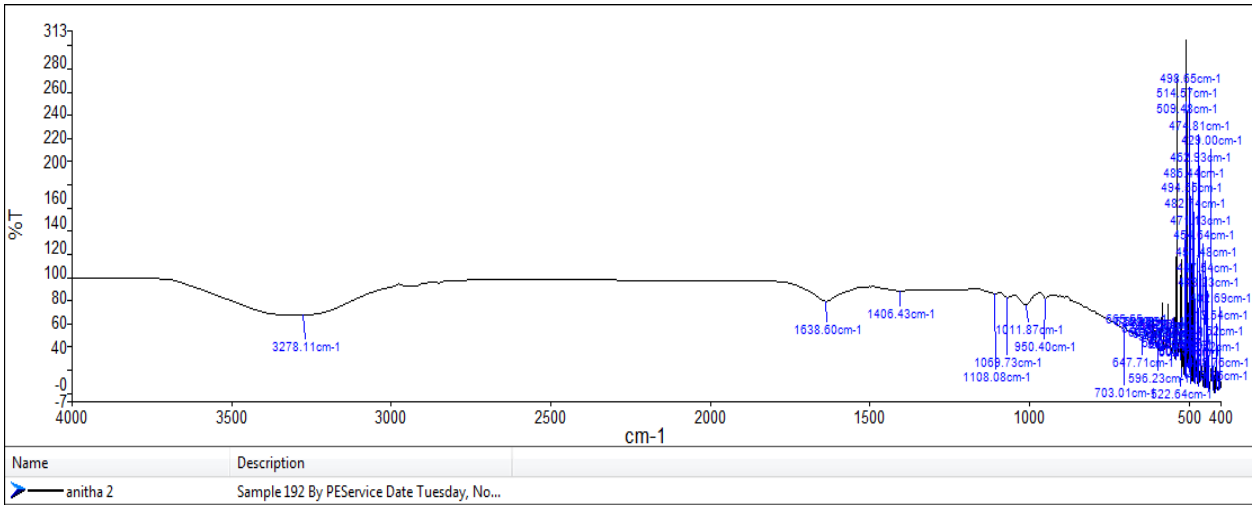

Fig 2: FT-IR spectrum of methanol extract of *B. monosperma* seeds

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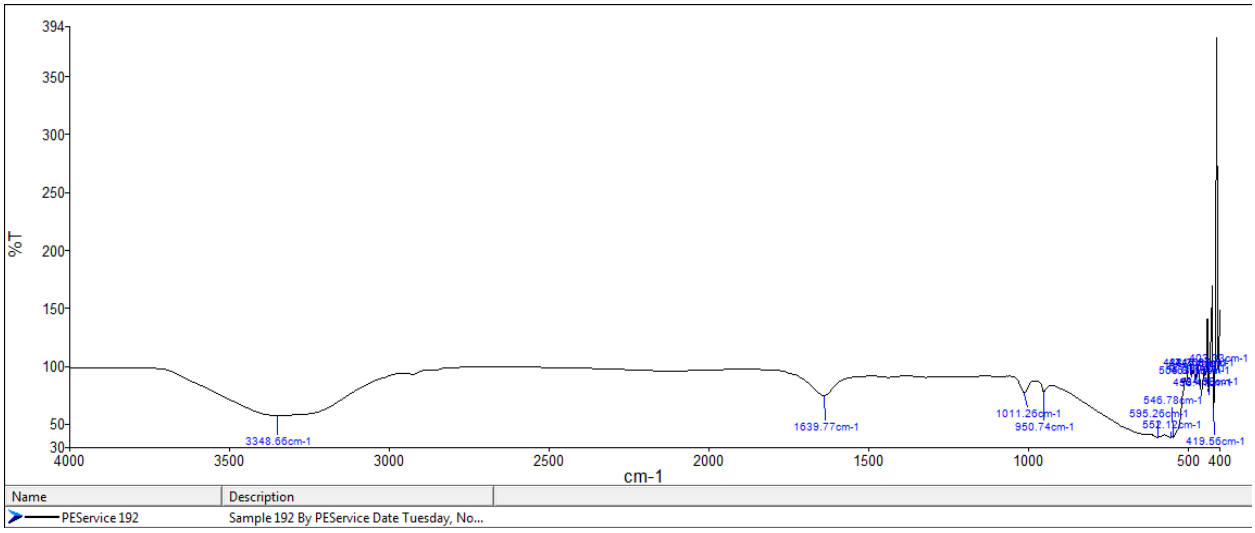

Fig 3: FT-IR spectrum of water extract of *B. monosperma* seeds

Different functional groups of the active ingredients present in seed extracts of *B. monosperma* were revealed by FTIR spectroscopic analysis in the form of peaks (Fig. 1-3). Characteristic frequency range 3277cm^{-1} corresponds to petroleum ether extract of *B. monosperma* representing OH stretch indicating the presence of alcohol and phenol, peak at 1616 cm^{-1} for C=C group and 1394.88 cm $^{-1}$ indicated C-H stretching, presence of alkanes. In methanol the characteristic bands were exhibited at 3278.11 and 1638.60 cm^{-1} indicating the occurrence of alcohols and aromatic compounds respectively. Peak value at 1406 cm^{-1} indicates the existence of nitro compounds. In water extract the presence of primary and secondary amines were indicated by the characteristic peak values at 3348 cm $^{-1}$. The peaks obtained at 1011, 1075 and 1069 cm $^{-1}$ from all the three extracts indicate the C-N stretching confirms the presence of aliphatic amines [27].

Among the functional groups obtained from the extracts, OH group was found to be consistently present in all the three extracts. The ability of hydrogen bond forming capacity of the OH group probably indicates the higher possibility of methanolic extract towards the microbial inhibitory activity. FTIR analysis of medicinal plants is used to confirm and detect the mix-substance systems such as traditional medicine and herbal medicine [28]. Phytochemical analysis of extracts by screening and FTIR revealed the presence of alcohols, phenols, sterols, carbohydrates, amino acids, amides and starch. Presence of all the functional groups mentioned in the seed extract indicates that the plant is possessing lot of medicinal properties.

CONCLUSIONS

Highest antimicrobial activity was observed in methanol seed extract of *Butea monosperma* against all the clinical strains tested. A remarkable point is that the extract has proved good microbial inhibitory action against gram positive bacteria followed by gram negative strains. Further research is needed to investigate the plant with more number of clinical strains to conclude its action against gram positive bacteria. This indicates that in future the drug can be used as a potent antibiotic agent against gram-positive bacteria and also other microbes. Through the FTIR study it is concluded that the phenols, alcohols and nitro compounds are the active antimicrobials present in *B. monosperma* seeds and in future, therapeutic antibiotics can be established if these active compounds of the extracts are isolated and characterized.

REFERENCES

[1] Christopher Dye, Ties Boerma, avid Evans, Anthony Harries, Christian Lienhardt, Joanne McManus, Tikki Pang, Robert Terry, Rony Zachariah. The world health report 2013: research for universal health coverage. Geneva. Switzerland; 2013.

[2] Cohen ML. Epidemiology of drug resistance: implications for a post antimicrobial era. Science 1992; 257: 1050-1055.

[3] World Health Organization. WHO Traditional Medicine Strategy 2002–2005, World Health Organization. Geneva. Switzerland; 2002.

[4] World Health Organization. Fact sheet No. 134. Geneva. Switzerland; 2008. [Online] Available from: http://who. Int / media entre/factsheets/fs134/en/. [Accessed on December, 2008].
[5] Loeraa JA, Reyes – Ortizb C, Kuoa YF. Predictors of complementary and alternative medicine use among older Mexican Americans. Complement Therap Clin Prac 2007; 13: 224-231.
[6] Shah PM. The need for new therapeutic agents: what is in pipeline? Clin Microbiol Infect 2005; 11: 36–42.
[7] Fabricant DS, Fansworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001; 109: 69–75.
[8] Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immune stimulatory compounds from *Andrographis paniculata*. J Ethnopharmacol 2004; 92: 291-295.
[9] Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phyto Res 2007; 21: 1142-1145.
[10] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. Council of Scientific and Industrial Research. New Delhi, 1956, pp. 330.
[11] Manas Kumar Das, Papiya Mitra Mazumder, Saumya Das, Sanjita Das. *Butea monosperma* (LAM.) kuntze – A comprehensive review. Int Res J Plant Sci 2011; 2: 215-219.
[12] Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A, Singh M, Misra RN, Dubey CB, Saxena KC. Management of giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. J Ethnopharmacol 1997; 56: 233–236.
[13] Manjulika yadav, sanjukta chatterji, sharad kumar gupta and geeta watal. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci 2014; 6: 539-542.
[14] Paritala Vikram, Kishore Kumar Chiruvella, Ilfah Husna Abdullah Ripain, Mohammed Arifullah. Medicinal properties of kesum (Polygonum minus Huds.). Asian Pac J Trop Biomed 2014; 4: 430-435.
[15] Sooad Al-Daihan, Manar Al-Faham, Nora Al-shawi, Rawan Almayman, Amal Brnawi, Seema zargar. Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. Journal of King Saud University – Science 2013; 25: 115–120.
[16] Mounyr Balouiri, Moulay Sadiki, Saad Koraichi Ibnsouda. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 2016; 6: 71–79.
[17] Apurv Gaur, Malarvili Ganeshan, Rameez Shah, Bholay AD. Determination of minimum inhibitory concentration of organic extract of *Catharanthus roseus* by a novel modified well diffusion technique. Int J Pure App Biosci 2016; 4: 177-182.
[18] Nithya devi J, Sivakumar R. Phytochemical Screening and GC-MS, FT-IR Analysis of Methanolic extract leaves of *Solanum torvum*. Int J Res Studies in Biosci 2015; 3: 61-66.
[19] Bhargava SK. Estrogenic and post coital anti conceptive in Rats of butin isolated from *Butea monosperma*. J Ethnopharmacol 1986; 18: 95- 101.
[20] Mehta BK, Dubey A, Bokadia MM and Mehta SC. Isolation and in vitro antimicrobial efficiency of *Butea monosperma* seed oil on human pathogenic bacteria and phyto pathogenic fungi. Acta Microbiological Hungry 1983; 30: 75-77.
[21] Divya fageria, Rao DV. A Review on *Butea monosperma* (Lam.) kuntze: A great therapeutic valuable leguminous plant. International Journal of Scientific and Research Publications 2015; 5: 1-9.
[22] El-Mahmood AM, Doughari JH, Chanji FJ. In vitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri.* Scientific Research and Essay 2008; 3: 102-105.
[23] Lin J, Opaku AR, Geheeb Keller M, Hutchings AD, Terblanche SE, Jäger AK. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol 1999; 68: 267-74.
[24] Shailendra SG, Vijay DG, Nanda kishore JD, Arun TP. Antimicrobial activity of *Butea monosperma* Lam. Gum. Iranian J Pharmacol 2008; 7: 21-24.
[25] Arvind R Suthar, Piyush J Vyas, Kartik V Vikani, Hiren P Vyas. Antibacterial activity of individual and combined extract of flowers of *B. monosperma* and *Cassia fistula*. J Pharm Chem Biol Sci 2016; 3: 573-577.
[26] Pooja Gupta and Naresh Chandra. Antibacterial study of *Butea monosperma* (LAMK.) TAUB. Plant parts using agar well diffusion method. World Journal of Pharmacy and Pharmaceutical Sciences 2016; 5: 1002-1010.
[27] Sumathi R, Anuradha R. FT-IR spectroscopic studies on flowers of *Allamanda neriifolia* Hook. Int J Curr Microbiol App Sci 2016; 5: 287-291.

[28] Liu HX, Sun SQ, Lv GH and Chen KK. Study on *Angelica* and its different extracts by Fourier Transform Infrared Spectroscopy and two dimensional correlation IR Spectroscopy. Bio Mol Spec 2006; 64: 321 - 326.