IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
February 2016 Vol.:2, Issue:4

Characterization and Screening of Antimicrobial Activity of Bioactive Compounds from Barks and Twigs of *Beautea monosperma* against Some Oral Pathogens

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Submission: 2 February 2016
Accepted: 9 February 2016
Published: 25 February 2016

Keywords: oral infections, *Beautea monosperma*, Gram-positive, Gram-negative, antimicrobial activity

ABSTRACT

Microbial resistance to presently available antimicrobial agents and their side effects has necessitated the search for new antimicrobial agent. Hence, here we searched for alternative and natural phytochemicals from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. We report here the antimicrobial effect of various extracts and isolated from *Beautea monosperma* on oral pathogenic strains by zone of inhibition assay *Beautea monosperma* barks and twigs extracts were evaluated for antimicrobial activity against some oral pathogenic strains of Gram-positive (*Streptococcus mutans, S.mitis* and *S. Sanguis)*, Gram-negative bacteria (*A.actinomycetemcomitans, P. gingivalis* and *B. forsythus*) and fungal stain (*Candida albicans*). Use of methanolic extract and isolated compounds from *Beautea monosperma* twigs and barks as a potential antimicrobial agent in prevention and treatment of oral infections and diseases has been suggested.

INTRODUCTION

World Health Organization (WHO) noted that most of the world's population depend on traditional medicine for primary healthcare. Plant extracts have been used for many thousands of years (1) in food preservation, pharmaceuticals, alternative medicine and natural therapies (2, 3). *Butea monosperma* is commonly known as Flame of forest, belongs to the family Fabaceae (4). *Butea monosperma* is one among four species belonging to the genus *Butea* Koenig, three species of which occur in India (5). It holds an important place because of its medicinal and other miscellaneous uses of economic value. Bark fibers are obtained from stem for making cordage (6). Stem bark powder is used to stupefy fishes. Young roots are used for making ropes (5). Green leaves are good fodder for domestic animals. Leaves are used for making platters, cups, bowls and beedi wrappers (5, 7). Leaves are also used for making Ghongda to protect from rains and are eaten by buffaloes and elephants. Tribals use flowers and young fruits as vegetables. Flowers are boiled in water to obtain a dye (4). Orange or red dye is used for colouring garments and for making skin antiseptic ointments (8). Fresh twigs are tied on horns of bullocks, on occasion of 'pola' and dry twigs are used to feed the sacred fire (4). In addition wood of the plant is mainly used for well-curbs and water scoop. It is also employed as a cheap board wood and for structural work, wood pulp is suitable for newsprint manufacturing (7).

Flower: Triterpene (9), butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, monospermoside (butein 3-e-D-glucoside) and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin) and steroids (12, 13).Gum: Tannins, mucilaginous material, pyrocatechin (7). Seed: Oil (yellow, tasteless), proteolytic and lypolytic enzymes, plant proteinase and polypeptidase. (Similar to yeast trypsin) (7). A nitrogenous acidic compound, along with palasonin is present in seeds (13). It also contains monospermoside (butein 3-e-D-glucoside) and somonospermoside. From seed coat, allophanic acid has been isolated and identified (12, 13). Resin: Jalaric esters I, II and laccijalaric esters III, IV.; Z- amyrin, e-sitosterone its glucoside and sucrose; lactone-nheneicosanoic acid-{-lactone (13,14). Sap: Chalcones, butein, butin, colourless isomeric flavanone and its glucosides, butrin (5).

Leaves: Glucoside, Kino-oil containing oleic and linoleic acid, palmitic and lignoceric acid (15). Bark: Kino-tannic acid, Gallic acid, pyrocatechin (15). The plant also contains palasitrin, and

major glycosides as butrin, alanind, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (-)-medicarpin, miroestrol, palasimide and shellolic acid (15, 16, 17, 18, 19, 20, 21, 22). Stem: 3-Z-hydroxyeuph-25-ene and 2,14-dihydroxy-11,12- dimethyl-8-oxo-octadec-11-enylcyclohexane (21). Stigmasterol-e-D-glucopyranoside and nonacosanoic acid (24). Stem: 3-Z-hydroxyeuph-25-ene and 2,14-dihydroxy-11,12- dimethyl-8-oxo-octadec-11-enylcyclohexane (23). Stigmasterol-e-D-glucopyranoside and nonacosanoic acid (24).

In the literature, *B. monosperma* is ascribed to have many medicinal properties. It has been used as tonic, astringent, aphrodisiac and diuretic. Its flowers are widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-implantation activity [25]. Roots of *B. monosperma* are reported to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumors. Pippali rasayana, an Indian Ayurvedic drug, employs *B. monosperma* and is used in the management of giardiasis [26]. The bark is reported to possess antitumor and antiulcer activities. The root bark is used as an aphrodisiac, analgesic and antihelmintic whereas the leaves possess antimicrobial property [27]. *B. monosperma* flowers are well-known antihepatotoxic principles of *B. monosperma* [29]. Gum is useful as astringent, depurative and useful in diarrhoea, haemorrhoids, haepoptysis, haematemesis, leprosy, skin diseases [30, 31]. Therefore, the plants have long since been deemed a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance to therapeutic treatments. Therefore, such plants should be investigated to better understand their properties, safety profiles and levels of efficiency against pathogenic microbes. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. [32]
The purpose of this work was therefore to evaluate antimicrobial activity of *Butea monosperma* barks on different microbial strains.

MATERIALS AND METHODS

Plant material (*Beautea monosperma*)

The plant has been selected on the basis of its availability and Folk use of the plant. A survey was conducted in the villages of Chhatarpur district and found that rural people of the villages of Chhatarpur rely on herbal remedies for their dental care. They are utilizing twigs (as tooth brush)

and barks (in powder form) of *Beautea monosperma* for their dental care without having any side effects of it. So this plant is selected for the study. (33)

Fresh twigs and barks of *Beutea monosperma* were collected from area adjoining forests of Bhopal in the month of March. Authentication of plant material (specimen voucher no. 864/21) was done by taxonomist Dr. Manjusa Saxena at department of botany, Govt. Maharaja College, Chhatarpur (M.P.).

Methanolic extraction

Plant material was subjected to hot continuous extraction with (500 ml) 95% methanol (30-40^{o}C) in a Soxhlet apparatus for 6 hours. The extraction procedure was ensured by pouring a few drops of extract from thimble left no residue on evaporation. After complete extraction, the solvent was evaporated and concentrated to dry residue. % yield was calculated for each extract after drying under vacuum. (34) Total ash value, acid insoluble ash, water soluble ash, and moisture content (loss on drying) of crude material was also determined. (35,36)

Estimation of Total Contents; Total phenolic content was determined by the modified Folin-Ciocalteu method while total tannin content was estimated by precipitating tannins with gelatin. (37)

Disk diffusion method: Screening of antimicrobial activity of extracts and standard drugs (chloramphenicol, gentamicin and fluconazole) was done by disk diffusion method. It was performed using 24 hours incubation (for bacterial culture) and 48 hours (for fungal culture) at 37^{o}C in 20 ml of agar medium. Bacterial and fungal inoculums were spread over the plates containing agar medium using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The extracts were dissolved in ethylene glycol and sterilized by filtration under aseptic conditions; empty sterilized discs (Whatman no. 5, 6mm diameter) were impregnated with 100µl of each of the extracts of different concentration and left to dry under laminar flow cabinet and placed on the agar surface. Paper disk moistened with ethylene glycol was placed on the seeded Petri dish as a vehicle control. Standard discs containing chloramphenicol (10µg/ml), gentamicin (10µg/ml) and fluconazole (10µg/ml) were used as reference control. All Petri dishes were sealed with sterile laboratory paraffin to avoid

contamination and eventual evaporation of the test samples. The dishes were left for 30 minutes at room temperature to allow the diffusion of test drugs and kept for incubation on 37^{o}C.(38, 39)

Fractionation of Methanolic extract of *B. monsperma*: Methanolic extract (10gm) of methanolic extract was taken and dissolved in small quantity of water which future is fractionated by extracting with pet. Ether (Fraction I), chloroform (Fraction II), acetone (Fraction III) and remaining aqueous fraction (Fraction IV) were collected and dried. All these fractions were subjected to chemical evaluation (Chemical Tests and TLC) for the identification of constituents. Then Screening of all fractions for antimicrobial activity by disc diffusion method was performed. TLC (Thin layer chromatography profile of acetone Fraction- III) of *Beautea monosperma*: Preparative TLC plates were used for chromatographic profile. Application of extracts and TLC of extracts: The extracts were applied with the help of microcapillary, just 2cm above from the bottom. The spots were equally sized, dried and developed.

Derivatizing agent: 5% Ferric chloride reagents.

Isolation of compound by column chromatography

Column size: Glass Column, 65 cms x 3 cms

Stationary Phase*:* Silica gel (60 to 120 mesh size)

Elution mode*:* Isocratic elution

Mobile Phase*:* Chloroform: Methanol: Glacial acetic acid

Identification of similar fractions*:* By TLC and derivatization with Ferric Chloride reagents.

Preparation of column: Column packing was done by wet packing method. Silica gel (activated at 105^{o} c) was taken, and suspended it in chloroform then transfers it in Column; allowed to settle down. At the top of silica layer, cotton plug was kept to avoid disturbance in silica layer during elution.

Identification of similar fractions: The different fractions of column chromatographic elution were monitored by TLC using UV-254 & 366nm chamber and derivatization with spray with $FeCl_3$ reagents. Fractions which show similar fingerprinting profile on TLC were collected and mixed and considered as one fraction. (40, 41)

Characterization of Isolated compound: Isolated compound was characterized by UV-absorption spectra, IR, NMR and Mass Spectra studies.

Minimum inhibitory concentration Assay: The agar dilution method recommended by the National Committee for Clinical Laboratory Standards was used. A series of two-fold microdilution of isolated fraction with ethylene glycol was prepared. Plates were dried at room temperature for 30 min prior to spot inoculation. Firstly plates were inoculated and then these plates were incubated at 37°C for 18-24 hours after that minimum inhibitory concentration was determined. Inhibition of bacterial growth in the plates containing test extract was judged by comparison with growth in blank control plates. The lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of incubation at 37°C was considered as Minimum inhibitory concentration. (42)

RESULTS AND DISCUSSION

Results obtained from preliminary phytochemical screening showed, Anthraquinones, flavonoids, terpenoids, tannins, resins and phenolic compounds are present in the methanolic extract of twigs and barks of *Beautea monosperma.*

Table no 1. Different Ash values of barks and twigs

S No	Total ash (%w/w)	Acid Insoluble Ash	Water soluble Ash (% w/w)
1	4.64%	2.08%	1.52%

Table no 2. Different Extractive values of *Beautea monosperma* barks and twigs.

S. No.	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)
1	12.20%	6.43%

Table no 3. Moisture content of *Beautea monosperma* barks and twigs

S. No.	Moisture content (% w/w)
1.	3.87%

Table no. 4. Total flavonoids content estimation (TFC) of *Beutea monosperma* extract

S. No.	Concentration (g/ml)	Absorbance
1	20	0.107
2	40	0.233
3	60	0.436
4	80	0.654
5	100	0.8
6	Sample	0.129

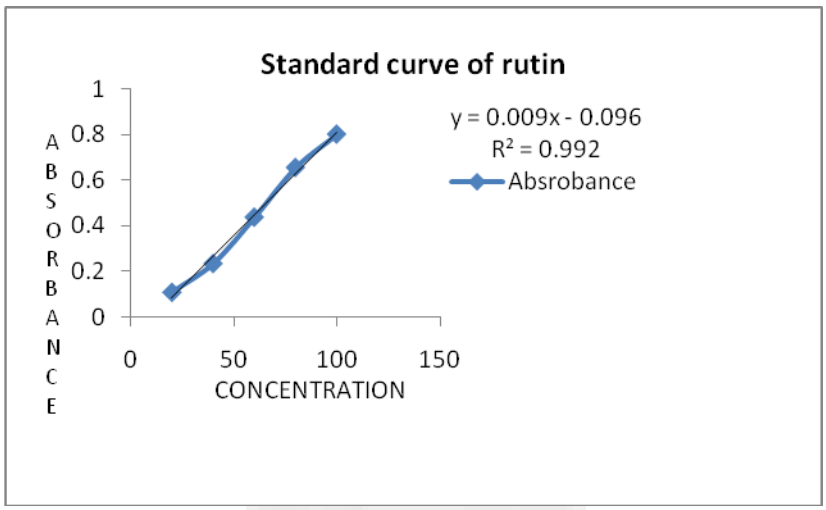


Fig. 1. Total flavonoid content estimation

Total flavonoids content was calculated as Rutin equivalent (mg/g) using the equation based on the calibration curve: Y=0.009X-0.096, R^2=0.992, where X is the absorbance and Y is the rutin equivalent (RE).

Table no 5. Estimation of Total flavonoids content (RE mg/gm of extract)

S. No	Extracts	Total Flavonoids (RE mg/ 1000 mg of dried extract)
1	Methanolic extract	2.54

Table no 6. Total Phenolic content estimation

S. No.	Concentration (g/ml)	Absorbance
1	25	0.375
2	50	0.688
3	75	0.910
4	100	1.105
5	150	1.468
6	200	1.798
7	Sample	1.287

Table no 7. Total phenolics content (TPC) in *B. monosperma* Barks and twinges extract

S. No	Extract	Absorbance	Total Phenol Content equivalent to gallic acid (mg/gm) of dried extract
1	Methanolic	1.287	14.57

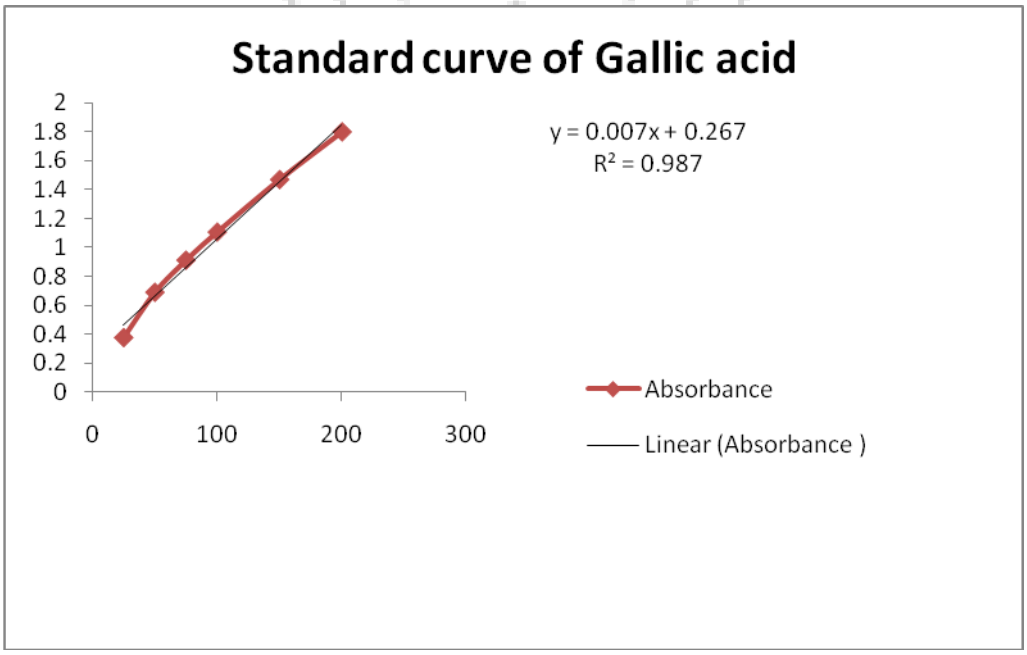


Fig 2. Standard curve of Gallic acid

Table no 8. Zone of inhibition for various concentrations of *Beautea monosperma* compared to reference drugs: activity against gram negative bacteria.

Micro-Organism → / Name of drug ↓	*A.actinomycetemcomitans*		*P. gingivalis*		*B. forsythus*	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 mg/ml)	17.67±1.47	100	16.88±0.87	100	19.34±0.68	100
Chloramphenicol (20 mg/ml)	16.33±0.33	92	10.45±0.87	61	11.56±0.63	59
MeOH Extract of *Beautea monosperma* (mg/ml)						
15	6.00± 00	00	6.00± 00	00	6.00± 00	00
30	6.00± 00	00	6.00± 00	00	6.00± 00	00
45	6.00± 00	00	8..44± 00	50	7.42±0.54	38
60	8.00± 00	45	9. 34±0.85*	55	8.20±0.52	42
90	9.36± 0.47*	52	9. 49±0.46*	56	9.89±0.38*	51
Control	6.00±00	00	6.00±00	00	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.

As the diameter of paper disc used was 6mm, 6mm diameter included in the table is indicative of no activity.

Percent was calculated after subtracting disc diameter (6mm) from all observations.

* indicates significant activity at $p<0.05$

Methanolic extract of *Beautea monosperma* have shown the significant activity against *A. actinomycetemcomitans* on the concentration 90 mg/ml, against *P. gingivalis* on the

concentration 60 and 90 mg/ml and against *B. forsythus*, it has shown significant activity on 90 mg/ml concentration. On the concentration of 15, 30 mg/ml, no zone of inhibition was observed.

Table no 9. Zone of inhibition for various concentrations of *Beautea monosperma* compared to reference drugs: activity against gram positive bacteria

Micro-Organism →	***Streptococcus mutans***		***S.mitis***		***S. sanguis***	
Name of drug ↓	**In mm Mean**	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 mg/ml)	18.00±0. 68	100	16.00±0. 68	100	17.00±0. 68	100
Chloramphenicol (20 mg/ml)	15.84 ±0.34	87	15.84 ±0.34	85	14.84 ±0.34	86
MeOH Extract of *Beautea monosperma* (mg/ml)						
15	6.00±00	00	6.00±00	00	6.00±00	00
30	6.30±0.32	35	6.30±0.32	35	6.00±0.30	34
45	8.00±0.85	44	8.00±0.22	43	8.40±0.85	42
60	9.54±0.64*	52	9.44±0.34*	51	8.54±0.12*	42
90	10.68±0.24*	59	10.66±0.20*	59	10.00±0.38*	58
Control	6.00±00	00	6.00±00	00	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.

As the diameter of paper disc used was 6mm, 6mm diameter included in the table is indicative of no activity.

Percent was calculated after subtracting disc diameter (6mm) from all observations.

* indicates significant activity at $p < 0.05$

Methanolic extract of *Beautea monosperma* have shown zone of inhibition 9.54 ± 0.64mm on 60mg/ml and 10.68 ± 0.24mm on 90mg/ml and have shown significant activity against *Streptococcus mutans* on the concentration 60 and 90mg/ml. On the concentration of 15 mg/ml, no zone of inhibition was observed. And inhibition was 52% and 59% as compared to the standard drugs.

Table no 10. Zone of inhibition for various concentrations of *Beautea monosperma* compared to reference drugs: activity against *Candida albicans.*

Micro- Organism → ↓ **Name of drug**	***C. albicans***	
	In mm **Mean**	**as** **%**
Fluconazole (10 mg/ml)	14.00±0.34	100
MeOH Extract of *Beautea monosperma* (mg/ml)		
15	6.00±00	00
30	6.00±00	00
45	6.80±00	48
60	8.46±0.48*	60
90	9.28±0.37*	66
Control	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.

As the diameter of paper disc used was 6mm, 6mm diameter included in the table is indicative of no activity.

Percent was calculated after subtracting disc diameter (6mm) from all observations.

* indicates significant activity at $p < 0.05$

Methanolic extract of *Beautea monosperma* have shown the significant activity on the concentration 60 and 90mg/ml and zone of inhibition 8.46±0.48mm (60% inhibition) and 9.28 ±

0.37mm (66% inhibition) respectively against *C. albicans* as compared to the standard drug fluconazole. On the concentration of 15and 30mg/ml, no zone of inhibition was observed.

Table no 11. Fractionation of Methanolic extract of *Beautea monosperma*

S. No.	Fractions	Yield (Mg)
1	Pet. Ether fraction-I	0.46
2	Chloroform Fraction-II	0.34
3	Acetone Fraction-III	6.25
4	Aqueous Fraction-IV	2.84

Screening of antimicrobial activity of various fractions:

Table no 12. Zone of inhibition of various fractions of *Beautea monosperma* compared to reference drugs activity against Gram-negative bacteria.

Micro-Organism → / **Name of drug ↓**	***A. actinomycetemcomitans* Zone of inhibition**		***P. gingivalis* Zone of inhibition**		***B. forsythus* Zone of inhibition**	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 ug/ml)	24.67±1.47	100	25.88±0.87	100	21.00±0.68	100
Chloramphenicol (10ug/ml)	22.33±0.33	95	9.45±0.87	38	8.56±0.63	31
Ciprofloxacin (10 ug/ml)	23.66±1.88	98	6.00±0.00	00	6.00±0.00	00
Erythromycin (10ug/ml)	16.00±0.44	62	6.42±0.64	00	6.00±0.52	00
Fraction –I	6.00± 00	00	7.00± 00	27	6.90± 00	20
Fraction-II	7.00± 00	33	7..00± 08	30	7.00±0.00	33
Fraction-III	14.84.± 0.62	61*	13.34±0.85	59*	10.66±0.52	51*
Fraction-IV	7.00 ± 00	20	7. 42±0.46	15	7.89±0.38	14
Control	6.00±00	00	6.00±00	00	6.00±00	00

Diameters of paper disc = 6mm, indicative of no activity, percentage was calculated after subtracting disc diameter (6mm).

Mean values shown in the table are mean of diameters of zone of inhibition taken with standard error.

Table no 13. Zone of inhibition of various fractions of *Beautea monosperma* compared to reference drugs: activity against Gram-positive bacteria.

Micro-Organism → Name of drug ↓	*Streptococcus mutans* Zone of inhibition		*S.mitis* Zone of inhibition		*S. sanguis* Zone of inhibition	
	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Gentamycin (10 ug/ml)	27.67±1.47	100	25.88±0.87	100	19.00±0.68	100
Chloramphenicol (10ug/ml)	25.33±0.33	94	24.45±0.87	95	17.56±0.63	84
Ciprofloxacin (10 ug/ml)	25.66±1.88	95	24.00±0.68	94	18.00±0.48	91
Erythromycin (10ug/ml)	15.40±0.44	62	15.42±0.64	60	13.00±0.52	61
Fraction –I	7.00± 00	34	6.90± 00	00	6.48± 00	20
Fraction-II	7.00± 00	34	6..80± 00	00	8.00±0.00	19
Fraction-III	12.66± 00	55*	11.34±0.85	36	16.20±0.52	81*
Fraction-IV	7.36± 0.47	25	7. 49±0.46	26	7.16±0.38	21
Control	6.00±00	00	6.00±00	00	6.00±00	00

Table no. 14. Zone of inhibition of various fractions of *Beautea monosperma* compared to reference drugs: activity against fungal strain.

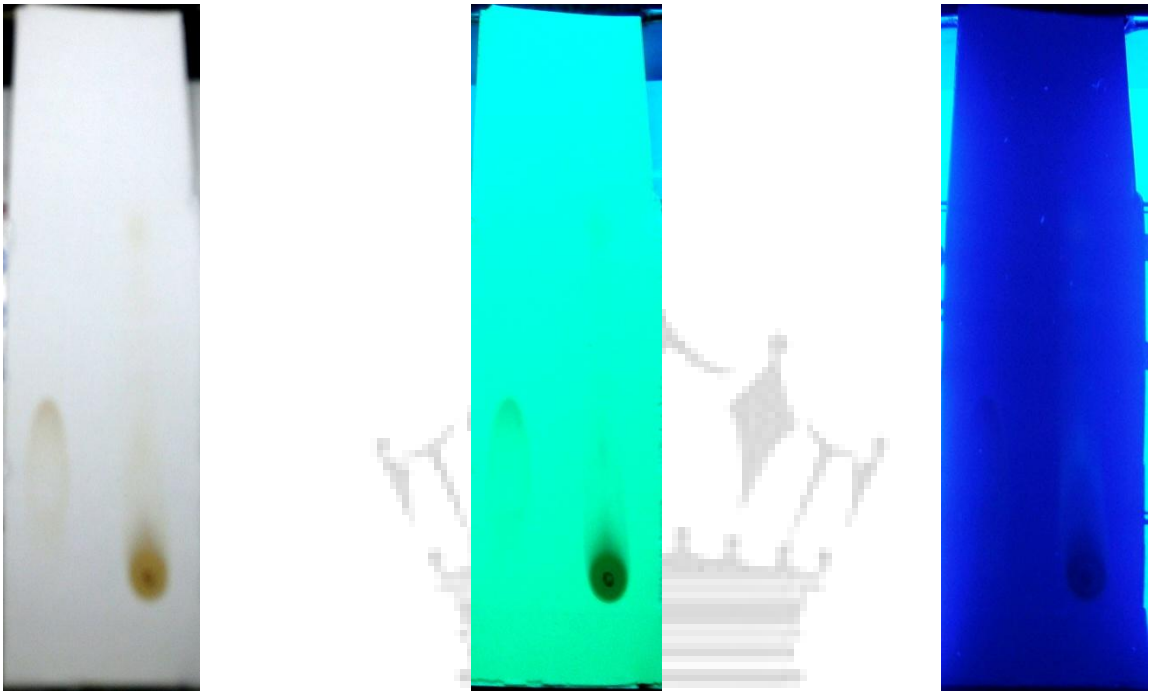
Micro- Organism → Name of drug ↓	*C. albicans* Zone of inhibition	
	In mm Mean	As %
Fluconazole (10 ug/ml)	27.38±1.22	100
Fraction –I	6.00± 00	00
Fraction-II	7.00± 00	25
Fraction-III	15.66± 00	67*
Fraction-IV	9.26±0.47	31
Control	6.00±00	00

Only acetone fraction (fraction-III) shown significant activity against gram positive, negative and fungal strains so only this fraction was considered for further study**.**

Rf value = distance traveled by solute/ distance traveled by solvent

3.5/ 6 = 0.583

Fraction no. 30-39 (Light Yellowish white color, 16.8mg) which shows green color after derivatization with $FeCl_3$ and also shows positive $FeCl_3$; these fractions were dried and subjected for the structural elucidation by spectroscopic techniques (UV Spectrophotometer), IR, NMR and Mass Spectra.



Normal light Short U.V. Long U.V

Fig no 3. Photograph of TLC of acetone fraction

Table no. 17. Characterization of isolated compound

S.No.	Characters	Remark
1.	Appearance	Amorphous Powder
2.	% Yield	0.021%
3.	Colour	Light yellow
4.	Solubility	Very soluble in water, benzene, chloroform, diethyl ether, ethanol
5.	Melting point	245-248^{0}C

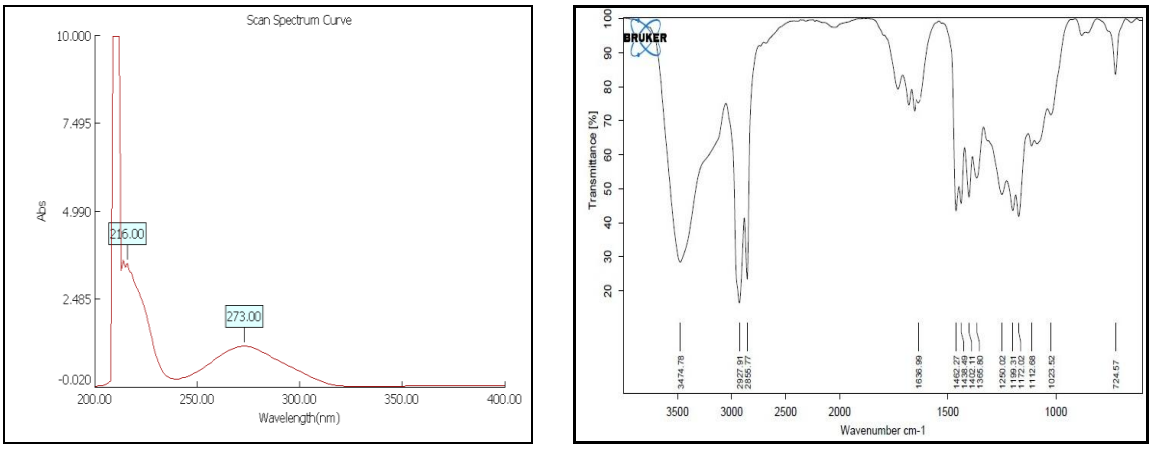


Fig no. 4. U.V. and IR spectra of isolated compound

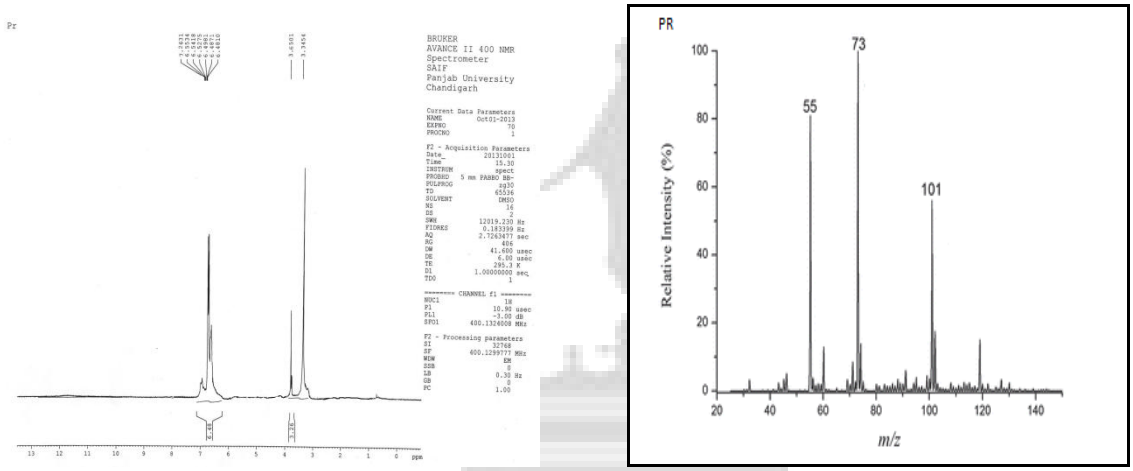
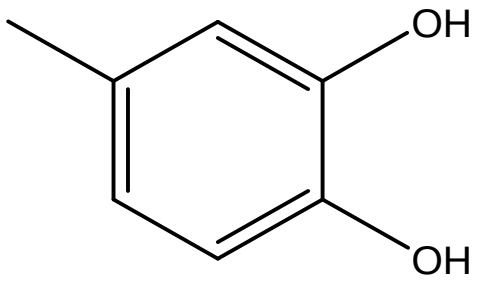


Fig no. 5. NMR and MASS spectra of isolated compound

IR interpretation: IR(KBr)cm^{-1} 3474.78, (OH, stretch), 3213(CH, stretch), 1662(C=C, stretch), 724.57 (Out of plan bending of aromatic H).

H1 NMR interpretation: H^{1}NMR(ppm); 6.55-7.26 (m, 2H Ar- OH), 6.48-6.54 (m, 3H Ar- H), 3.65 (m, 3H Al-H), 3.34 (m, DMSO)

Mass interpretation: MASS (m/z %): 117 (Base peak).



4-methylbenzene-1,2-diol

All the spectral data shows that the isolated compound is 4-methylbenzene -1,2-diol, (a pyrocatechol derivative).

Table no 18. Minimum inhibitory concentration of fraction-III (acetone) on Gram negative bacteria with gentamycin as standard reference.

Micro-Organism →	***A. actinomycetemcomitans* Zone of inhibition**		***P. Gingivalis* Zone of inhibition**		***B. forsythus* Zone of inhibition**	
Name of drug ↓	**In mm Mean**	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 ug/ml)	24..27±1.57	100	24.28±0.37	100	21.24±0.62	100
Fraction 1/10 dilution of fraction-III	13.36± 0.4 13.00± 00	50* 46	11.49±0.46 9. 34±0.85	45 30	9.89±0.38 9.20±0.52	20 22
1/100 dilution of Fraction-III	8.00± 00	11	8. 40±0.46	20	9.00±0.38	21
Control	6.00±00	00	6.00±00	00	6.00±00	00

Table no. 19. Minimum inhibitory concentration of fraction III (acetone) on Gram-positive bacteria with gentamycin as standard reference.

Micro- Organism → **Name of drug ↓**	***Streptococcus mutans*** **Zone of inhibition**		***S.mitis*** **Zone of inhibition**		***S. sanguis*** **Zone of inhibition**	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin(10ug/ml)	26.67±1.47	100	23.88±0.87	100	19.34±0.68	100
Fraction-III	13.66.00±0.47	46	10.49±0.46	44	8.89±0.38	90
1/10 dilution of fraction-III	11.00± 00	38	9. 34±0.85	39	16.20±0.52	78
1/100 dilution of Fraction-III	8.00± 00	26	7. 49±0.46	31	15.89±0.38	73
Control	6.00±00	00	6.00±00	00	6.00±00	00

MIC (Minimum inhibitory concentration) was studied for acetone fraction (fraction-III) and results were compared with standard antibiotics. It was observed dilution altered activity gradually. In gram positive bacteria (*Streptococcus mutans)* to 80% and 60% at 1/10 and 1/100 dilutions respectively. In *S. sanguis*, activity decreased with 1/10 and 1/100 dilutions to 87% and 80% respectively. *S. mitis* also had shown decrease in activity with dilutions. Gram negative bacteria (*A. actinomycetemcomitans P. gingivalis* and *B. forsythus)* also showed decreased activity with dilutions *A. actinomycetemcomitans* showed only 11% inhibition at 1/10 dilution. While activity of fraction –III against *B. forsythus* was not affected by dilutions. Further more acetone fraction (fraction-III) is more effective against gram positive compared to gram negative. However, significant activity ($p < 0.05$) is shown in all gram positive, negative as well as fungal strains. (83% inhibition against *S. sanguis*, 50% inhibition against *A. actynomycetemcomitans* and 71% inhibition against *C. albicans* respectively). Fraction-III showed comparable activity to the standard antibiotics. It has shown the highest inhibition in *S. Sanguis* (83% inhibition) and compared to standard antibiotics. *Candida albicans* has shown 71% inhibition against fluconazole as standard antibiotic. While fraction-III showed significant ($p < 0.05$) activity against *P. gingivalis* and *A actinomycetemcomitans*, 50% and 48% inhibition respectively.

CONCLUSION

The present study suggests that acetone fraction from barks and twigs of *Beautea monosperma* possesses significant antimicrobial activity at very low concentration (20ug/disc) on oral pathogenic bacteria. The use of acetone fraction of the extract from barks and twigs as a potential antimicrobial agent in prevention of oral infections and diseases has been suggested.

ACKNOWLEDGEMENTS

We thank Mr. Ashish Kesharwani from Cipla ltd. Mumbai for procuring us different antibiotics. We also like to thank Hi-Media lab and All India Institute of Medical sciences, New Delhi for providing us culture media and microbial strains to conduct this research work

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