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Original Research Article

Evaluation of Plant Secondary Metabolites Composition and Antimicrobial Activities of *Eucalyptus globulus* Extracts

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Keywords

Phytoactive compounds, Phytochemical Analysis, Antibacterial activity

A B S T R A C T

The objective of present study was to investigate the antimicrobial and phytochemical appraisal of eucalyptus globules plants. Four parts (leaf, stem bark, root, flower and fruit) of this plant Organic (petroleum ether) and aqueous (distilled water) solvent extract of *Eucalyputsglobulus* were play consequential antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and salmonella typhi*. The antibacterial potential of petroleum ether extract of *Eucalyptus* was tested by using disk well diffusion method. Phytochemical tests were accomplish gave purposeful results that the antibacterial activity of plant *Eucalyptus* was due to the presence of phytoactive compounds like steroids, phenolic compounds, tannins, flavonoids and saponins, alkaloids. The study includes physicochemical evaluation, preliminary phytochemical screening and antimicrobial activity.

Introduction

Eucalyptus is a diverse genus of flowering trees and shrubs. It is native to Australia and occur in Tasmania as large evergreen, and the genus *Eucalyptus* contains about 600 species. Tree can grow to 375-480 feet (125-160 meters); The Aborigines (native Australians) have traditionally used *Eucalyptus* leaves to heal wounds and fungal infections 1.Plants chemicals plays important role against including fungi and herbivorous mammals presence of phytochemical in whole parts of the plants all plants parts have different type of secondary metabolites that is good source of antimicrobial, antioxidant and antidiabetic known active compounds are 12,000 in plants science. Chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines phytochemicals work as same manner. It can be metabolize in body easily containing no side effect neither costly and easily available in simply word we have explained "no more cost and no lost" and herbal plant behavior just like conventional drugs. Eucalyptus posses versatile medicinal characteristics, that have insulin mimic property. Many of drugs of established therapeutic value used in the pharmacopeias of different countries grow in many parts of Sudan. Bearing in mind all this, the main task of this study is to spot light on the anti-hyperglycemic effects of the veryimportant medicinal tropical plant which is known as *Eucalyptus camaldulensis Dehnh* (Sanii, 2014).

The River Red Gum (*Eucalyptus camaldulensis*) is a tree of the genus Eucalyptus, its one of around 800 in the genus and it is a plantation species in many parts of theworld, but it is native to Australia, where it is widespread, especially beside inland water courses (Slee, 2007). Eucalyptus Leaves containing 0.9–1.2% oil known active compounds in eucalyptus leaf part are Cineole, Pinene Sesquiterpene alcohols eudesmal, a-phellandrene, Astrgin and its traditional uses Antiseptic, infections of upper respiratory tract, skin diseases, burns, rheumatism.

Researchers adapt experimental methods to better represent possible future applications in their particular field. However, since the outcome of a test can be affected by factors such as the method used to extract the EO from plant material, the volume of inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature comparison of published data is complicated (Rios *et al.,* 1988).

A number of reviewers have surveyed the methods used for antibacterial activity studies carried out with Eos (Friedman, *et al.,* 2002 and Koedam, A., 1976). In papers published since, the number of variations on culture medium, size of inoculums, choice of emulsifier and basic test method has further increased.

In this studies we are expected that plant extracts showing antimicrobial properties of extracts that have saponin,tannin, glycosides,alkaloids, flavonoids, terpenoidsfrom *E. globulus* that showed significant growth inhibition for salmonella, sthyplococcus, E coli and total bacterial counts tested whose the problem relates to the emergence of strains that possess multiple resistances to a range of antibiotics, thereby making them difficult to treat These derivatives could be potential control of clinical pathogenic bacteria

The encouraging results indicate the *E. globules* might be exploited as natural antibiotic for the treatment of several infectious diseases caused by this bacterium, and it were useful in understanding the relations between traditional cures and a production of those antibiotics that have insulin mimic property and traditional therapeutics drug.

Materials and Methods

Plant material

The plant specimens (leaves, root and stem bark,Fruit of *Eucalyptus globules* for the present study were collected from university campus at M.G.C.G.V., Chitrakoot, Satna, MP, India and authenticated by.

A voucher specimen no.103 is kept at Herbarium of Research Lab, M.G.C.G.V. Chitrakoot for further reference. The All chemicals and reagents used including the solvents were of analytical grade.

Preparation of extracts

All the plant specimens were washed thoroughly with normal tap water followed by sterile distill water and air dried at room temperature for one week to get consistent weight. The dried samples were crushed to powder using grinder. Powder were stored in air tight container bottle 10 gm of each sample were extracted with 100 ml of methanol and water for using soxhlet extraction. The mixtures were evaporating under reduced pressure using rotary vaccum evaporator (Buchi type) and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals

Quantitative yield of extract:

The yield of resultant plant extrats were found by following formula:

All the obtained extracts were then subjected to different qualitative tests to find out the presence of specific phytochemicals (Tripathi and Tripathi 2017).

$$\text{Percentage yield} = \frac{\text{Yield of the sample extract Obtained (g)} \times 100}{\text{Weight of the powdered sampled used (g)}}$$

Physicochemical Analysis

Physicochemical standardization which includes extractive values in different solvents, totalash value, acid insoluble ash value, loss on drying, pH values(1% and 10% solutions)were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia Kokate *et al.,* 1994.

Preliminary Phytochemical Screening

The physiochemical investigation of the petroleum ether and water extracts of *eucalyptus globulus*was carried out with standard procedures (Harborne, *et al.,* and Sofowara *et al.,*) for determining thepresence and/or absence of phytochemicals.

Preliminary phytochemical screening

Determination of total polyphenol

The concentration of phenolics in methanol fraction of *Syzygium cumini* (L.) was determined with the Folin-Ciocalteu's reagent (FCR) using specrophotometric method (Lowry *et al.,* 1951, Dwivedi *et al.,* 2017). Each sample (1 ml) was mixed with 0.5mL FCR (diluted 1:10, v/v) followed by 2 ml of sodium carbonate (20.00%, v/v) solution. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 ug/ml) were prepared. The absorbance was measured at 660 nm for test and standard solutions against the reagent blank at 765nm after incubation at room temperature for 90 min. Results were expressed as mg of GAE/gm of extract.

Determination of total proteins

Extraction of Protein from sample

Extraction is carried out with buffers used for the enzyme assay. Accurately weighed 500 mg of the sample were ground well with a pistle and mortar in 5-10 ml of the buffer. Centrifuged (SORVALL RC 5B plus) 20 minutes at 10,000 per rpm and collected supernatant used for protein estimation.

Standard Protein solution

Weighed accurately 50 mg of bovine serum albumin (fraction V) and dissolved in distilled water and the volume was made up to 50 ml in a standard flask. Diluted 10 ml of the stock solution to 50 ml with distill water in a standard flask. 1 ml of this solution contains 200 µg protein.

Estimation of protein

Pipette out 0.2, 0.4, 0.6, 08 and 1 ml of the working standard into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes. The volume was made to 1ml in all the test tubes. A tube with 1 ml of water serves as the blank. Added 5 ml of Alkaline copper solution to each tube including the blank. Mixed well and allowed standing for 10 minutes. Then added 0.5 ml of freshly prepared Folin-Ciocalteau reagent mixed well and incubated at room

temperature. Kept in the dark for 30 min and blue color is developed. The absorbance was measured at 660 nm by UV spectrophotometer and calculated the amount protein in the sample. By plotting a standard graph and expressed the amount of protein in mg/gm of sample.

Determination of Total Saponin

Determination of total saponin was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponin estimation 500 µl of sample, 500 µl of 0.5% anisaldehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60 °C. After 10 min tubes were cooled and absorbance was taken at 435 nm by UV spectrophotometer. The amount of saponin was calculated as saponin equivalent from the calibration curve of standard saponin (100-1000 µg/ml) (Khandelwal *et al.,* 2003 and Sreevidya, *et al.,* 2003).

Determination of alkaloids

Calibration curve

The calibration curve was obtained with bismuth nitrate pentahydrate stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of stock solution into separate 10ml standard flask and diluting to volume with distilled water. A 1ml amount of this solution was taken and 5ml thiourea solution was added to it the absorbance value of the yellow solution was measured at 435 nm against colorless reagent blanks.

Assay for alkaloids

A 5 ml amount of the extract/solution was taken and the pH was maintained at 2-2.5 with dilute HCL. A 2 ml amount of Dragendorff's Reagent was added to it, and the precipitate formed was centrifuged. The centrifuged was checked for complete precipitation by adding DR. after centrifugation the centrifuge was decanted completely and meticulously. The precipitate was further washed with alcohol. The titrate was discarded and the residue was then treated with 2ml disodium sulfide solution. The brownish black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2mlconcentrate nitric acid with warming if necessary this solution was diluted to 10 ml in a standard flask with distilled water.1ml was then pipetted out and 5 ml thiourea solution was added to it, the absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated using calibration curve. The amount of bismuth corresponds to the amount of alkaloids present.

Antimicrobial activity

Sample preparation

Take 10gm of sample and extract the sample with 95% methanol and pure double distilled water (100ml) through Soxhlet method for 6hrsandcontinuous heating apply.

After 6 hrs, the extract were collected and place them on vaccum evaporator until the complete dryness. After the complete dryness extract were scratch carefully and preserve them for antibacterial activity.

Preparation of inoculums

The inoculums was prepared from active pure culture of isolated cells and these were diluted with 10ml of sterile distilled water and place the culture in hot air incubator for

24 test with again culture were diluted with again 10 ml of distilled water.

Screening of Antibacterial Activity

Bacteria tested

All out of the four important bacterial strains we usually preferred i.e. *E. coli*, staphylococcus, pseudomonas, and salmonella throughout this investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Rani Durgawati Vishwavidyalya Jabalpur India. The young bacterial broth cultures were prepared before the screening procedure.

Antibacterial susceptibility test

The disc diffusion method was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using different media in for different pathogen Cetramide agar base, mannitol salt agar obtained from Himedia (Mumbai). The Cetramide agar base plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 1ml inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes.

The test sample of concentration 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37 °C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic streptomycin of concentration 1mg/ml was used as positive control.

Results and Discussion

In present study the leaves, stem bark and root of *Eucalyptus globules* (L) were evaluated for its physicochemical and phytochemical, antimicrobial aspects. Organoleptic parameters revealed that the powder of leaves, stem bark, fruit, flower and root of *eucalyptus globules* L are green, brown and reddish brown, brown in color, with the characteristic odour, bitter taste and fine and hard texture (Table-1). Physicochemical investigations for all parts of drug powder were performed for moisture content, ash content, acid insoluble ash, water soluble extractive; alcohol soluble extractive, pH and the results were tabulated in (Table-2). The results are expressed as mean (n=3) ± Standard deviation (SD). The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of drug. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards [20]. The extractive values names water soluble and alcohol soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. pH of 10% w/v solution revealed that the formulations are acidic. The less value of moisture content could prevent bacterial, fungal or yeast growth. The results of preliminary phytochemical screening in the methanolic and water extracts of the drugs showed the presence of carbohydrates, flavonoids, flavanol, resin, saponins and tannins (Table -3) which could make the drug useful for treating different ailment as having a potential of providing useful drugs for human use.

Table.1 Organoleptic Characters

S.N	Parameters	Observation				
		Leaves	**Stem bark**	**Root**	**fruit**	**flower**
1	Color	Green	Dark brown	Reddish brown	brown	brown
2	odour	Characteristic	Characteristic	Characteric	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter	-	-
5	Texture	Fine	Hard	Hard	-	-

= no identification

Table.2 Physicochemical parameters of *Eucaluptus globulus*

S.N.	Parameters	Value (n=3)				
		Leaves	**Stem**	**Root**	**Fruit**	**flower**
1	Loss on drying at 105^0C (%w/w)	5.07	8.00	6.22	7.1	3.0
2	Total ash value (%w/w)	6.29	4.92	4.12	5.34	2.33
3	Acid-insoluble ash value (%w/w)	5.33	3.06	3.62	4.76	3.23
4	Water soluble extractive value (%w/w)	17.2	18.23	17.52	17.52	4.56
5	Alcohol soluble extractive value (%w/w)	12.35	12.66	10.78	11.92	8.43
6	pH (Filter of 10% w/v aqueous solution)	4.77	4.9	5.6	5.7	6.0

*****The results are expressed as mean (n=3) ± Standard deviation (SD)

Table.3 Phytochemical Analysis

Phytochemicals	Tests	Observation							
		Leaves		**Stem bark**		**Root**		**fruit**	**flower**
		Ethanol extract	Aqueous Extract	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	methanol extract	Aqueous extract
Carbohydrates	Anthrone test, Fehling test molish	+ + +	+ + +	+ ++	+ ++	+ ++	+ + +	+ +	_ +
Resin	Acetone	+	+	+	+	+	+	-	-
Saponins	Foam tests	-	+	-	+	-	+	-	-
Alkaloids	Dragendorff's test, wagners test	- -	_ _	- -	_ -	- -	_ -	+	+
Steroids	Salkowski tests	+	+	+	+	+	+	+	+
Flavanoids	Shinoda test	-	-	-	-	+	+	+	+
Tannins	5% $FeCl_3$	-	+	+					
Amino acids and Proteins	Biurate test	-	-	-	-	-	-		

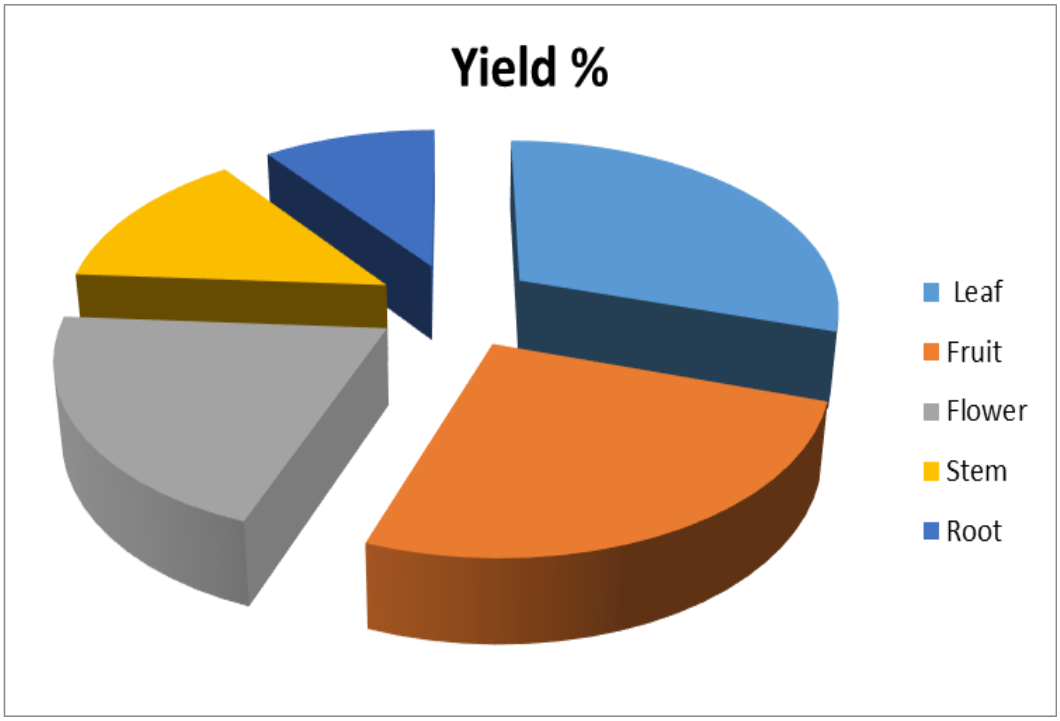
Table.4 Quantitative estimation of phytochemical analysis

SN.	Phytochemical	Concentration inug/ml					Unit
		leaf	Stem bark	fruit	flower	Root	
1	Polyphenol	1.014	1.1251	4.766	6.342 and	2.1569 ug/ml	ug of GAE/ml of extract
2	Protein	2.3569	3.5166	2.001	1.381and 9.8578	2.3569	ug of BSA/ml of extract
3	Saponin	4.5314	2.9930	8.345	11.332	4.5314	ug of Saponin/ml of extract
4	Alkaloid	24.499	15.4782	11.6926	14.662	6.453	ug of Bismuth nitrate/ml of extract
5	flavanoids	22.499	15.4667	11.6926	15.654	13.891	

Antibacterial zone of inhibition in mm

Test organism	streptomycin	leaf	Stem bark	fruit	flower	root
E. coli	**18**	**15**	**16**	**14**	**13**	**11**
s. aureus	**22**	**20**	**19**	**11**	**10**	**10**
S. typhi	**23**	**18**	**16**	**16**	**13**	**9**
Pseudomonas	**23**	**17**	**22**	**13**	**17**	**7**

Fig.1 Yield % of different plant parts of *Eucalyptus globules*



W / W

Physicochemical analysis

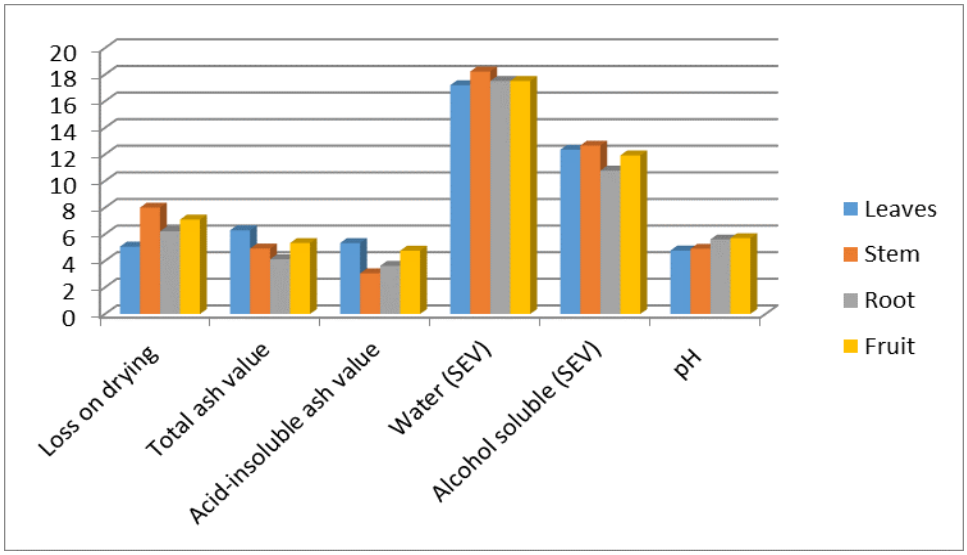


Fig.1 Calibration curve for protein

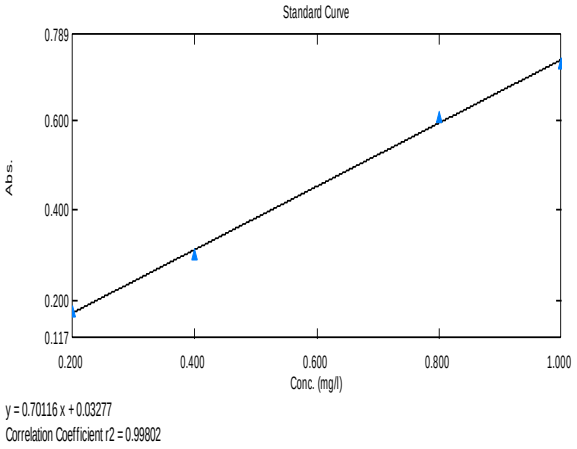


Fig.2 Calibration curve for saponin

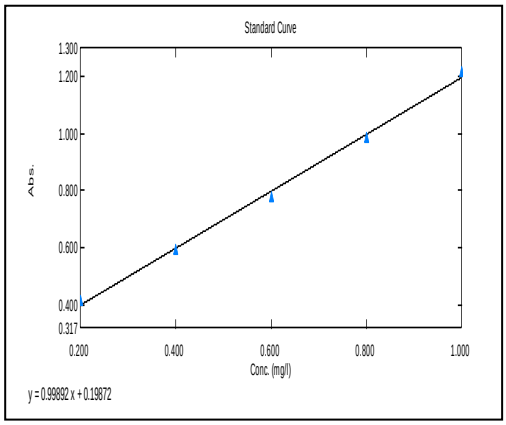


Fig.3 Calibration curve for alkaloids

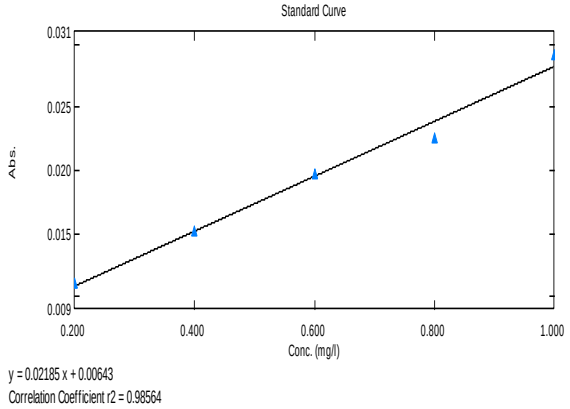
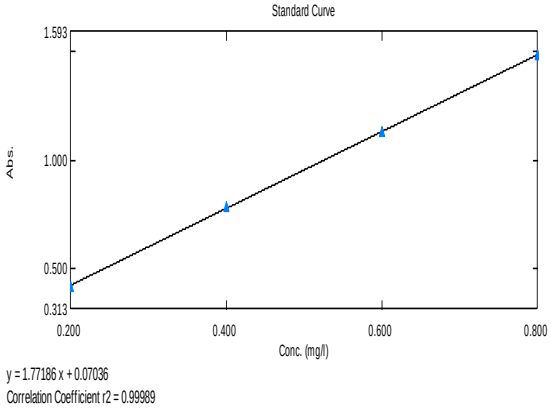


Fig.4 Calibration curve for total polyphenol



Calibration curve for standard phyto-chemical

The quantitative analysis of phenolic contents, protein, saponin and alkaloid were carried out using spectrophotometer and results are given in (Table-4). The total phenolic contents in the examined plant extracts is expressed in terms of gallic acid equivalent (the standard curve equation: Y=1.77186x+0.07036, r2=0.99989).the concentration of total phenols was measured 1.014, 1.1251,4.766,6.342 and 2.1569 ug/ml for leaf, stem bark, fruit, flower and root respectively. The protein contents in the examined plant extracts is expressed in terms of BSA equivalent (the standard curve equation: Y=0.05346x + 0.1614, r2=0.99843).The concentration of protein was measured 2.3569, 3.5166, 2.001,1.381 and 9.8578 ug/ml for leaf, stem bark and root respectively (Chopra *et al.,* 1992). The saponin contents is expressed in terms of saponin equivalent (the standard curve equation: Y=0.07150x + 0.01277, r2=0.97804).The concentration of saponin was found 4.5314, 2.9930,8.345,11.332 and 12.6993 ug/ml for leaf, stem bark, fruit flower and root respectively. Similarly the alkaloid contents is expressed in terms of mg of Bismuth nitrate/g of extract equivalent (the standard curve equation: Y=0.99892x + 0.19872, r^2=0.99693). The concentration of saponin was found 24.499, 15.4782, 11.6926, 14,672, 15.233 ug/ml for leaf, stem bark, fruit, flower and root respectively.

Similarly the flavanoid contents is expressed in terms of mg of quercetin /g of extract equivalent (the standard curve equation: Y=0.99892x + 0.19872, r^2=0.99693).The concentration of flavonoids was found 22.499, 15.4667, 11.6926, 15.654, 13.891 ug/ml for leaf, stem bark, fruit, flower and root respectively.

This investigation has shown that the extracts of *E. globules* contain antimicrobial capability found to be effective against pathogenic microorganisms involved in wounds infections, typhoid fever gastrointestinal tract infections and urinary tract infections. Therefore, the results of this study provide a use of the different plant parts in traditional medicine. The activities of *E. globules* should further be investigated against wide range of microorganisms. Studies should be carried out with a view of sourcing antimicrobial agents for drug development.

The calculation drawn the above studied on the basis of zone of in the antibacterial inhibition and different solvent system showed that activity of leaf and stem bark of eucalyptus have found to be more active against staphylococcus. The antibiotics i.e. streptomycin showed maximum zone against particular plant and pathogen (Takahashi *et al.,* 2004).

The current findings can be used as a reference standard for identification of genuine drug with same ingredients. Further marker based identification and pharmacological studies enhance its therapeutic potential to maintain Diabetes Mellitus.

The present study was carried out as per WHO/Ayurvedic pharmacopoeial standards for various standardization parameters such as organoleptic, physiochemical parameters like total ash, acid insoluble ash, water & petroleum ether soluble extractive values, and loss on drying, phytochemical analysis. Presence of various phyto-constituents can serve as basis for screening of different pharmacological activities, investigation and further research. Total phenol, protein, alkaloid and saponin contents were investigated in the drug. The pharmacological action of these plant drugs will be determined by the nature of these

chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects herbal drug standardization for the proper identification of medicinal plants. So, above result of leaf and stem bark posses more zone of inhibition against pathogen we found that if we will use eucalyptus herbal medicine in our plant drug, it increases the immunity of rural and tribals people which is easily available at a cheap level and finally these further clinical that is required for their future prospective The study of these parameters will be boosting the research in the development of herbal medicine for combat diabetes and antibacterial activity.

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