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Biochemical assesment of leaves and stem bark of *Bridelia montana* (Roxb). willd

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Abstract
*Bridelia montana (*ROXB) WILLD. is a large shrub or small tree usually growing up to ten meters tall but occasionally upto twenty meters. In present work highest amount of Nitrogen (2.61%) & Phosphorus (0.35%) were found in leaves and higher amount of Potassium (1.32%), Calcium (0.76%) and Magnesium (0.47%) were recorded in stem bark respectively. In the present study we found that, ash (20 %), dry matter (59.5%), and crude Protein (16.31 %) content were more in the leaves and crude fiber (40%), crude fat (65.4%) and moisture (65.7%) were more in stem bark. The acetone, alcohol and distilled water extract shows highest phytochemicals than that of the others. In fresh plant antioxidant analysis leaves contain high amount of carotenoid (6.86mg/100gm) and stem bark shows higher amount of polyphenol (19.52mg/100gm) and ascorbic acid (20.8mg/100mg) content. Antioxidant analysis of dry powder it was found that maximum antioxidant potential present in acetone extract as compare to other solvent extract. Antimicrobial activity was found highest in leaves compare to the bark.

Keywords: *B. montana*, antimicrobial, phytochemicals, nutraceutical analysis, etc

Introduction

For the protection, plant produces secondary metabolites are nothing but the preliminary phytochemicals. In the various diseases management process, Phytomedicines or Phytotherapeutic agents which are standardized herbal preparations consisting of complex mixture of one or more plants was used in most of the countries [58]. According to WHO definition, active biomolecules of plant parts or plant materials in the crude or processed state and certain excipients like solvents, diluents or preservatives are present in the herbal drug [53].

The genus *Bridelia* includes 60 species, spread over the tropics and subtropics of the old world. According them, some species are used in medicines. The genus *Bridelia* is rich in tannin and phenolic compounds which are associated with various pharmacological activities. *B. montana (ROXB) WILLD* is a large shrub or densely branched shrub usually growing up to 10 meters tall but occasionally up to 20 meters, found in granite or basalt derived sandy soil. The bark is used for tanning, as a liniment with Gingelly oil in treating rheumatism, for the removal of urinary concretions, astringent. The leaves are used as cattle-fodder [32]. *B.montana* Willd. and *B. retusa* Spreng. are used in indigenous medicines [21, 11].

Domesticated foods in combination with wild edible species has remained hallmark of many African agro-pastoral societies [2, 22, 23, 24]. Antimicrobial agents or antibiotic are more resistance against the most of the microorganisms. So discovery of that new antimicrobial agent is essential in 21st century [1, 4]. Plants are rich source of many potent and powerful drug as well as antimicrobial agent [53]. Abundant amount of bioactive compounds with rich source of nutrients especially dietary fibers, vitamins, and minerals was present in wild and underutilized fruits [29, 34, 42]. Studies on nutritional and phytochemical status and exploitation of wild fruits are much helpful for overcoming food security problems [40] and medicinal problems.

Reactive nitrogen species and reactive oxygen species are continuously produced in the body and are neutralized and eliminated by many endogenous mechanisms [62]. Antioxidant are chemical compounds which bind to free oxygen radicals and prevents these radicals from damaging healthy cells. For the nutralization of that reactive species antioxidant are very much helpful. A large number of biochemical are biosynthesized by the plants and for that they may be considered as most important source of chemical compounds necessary for the pharmaceutical industry. The bark is used as a liniment with Gingelly oil in treating rheumatism, for the removal of urinary concretions (Ayurveda), astringent. The leaves are used as cattle-fodder [32]. As there is a paucity of data on the nutraceautical and antimicrobial study of leaves and stem bark of *B. montana*, the present was aimed to examine the biochemical composition and nutraceutical value of leaves and stem bark. Additionally we analysed the preliminary phytochemical compounds and antimicrobial activity of leaves and stem bark methanolic extract of *B. montana*.

Material and Methods

Collection and Preparation of plant material

The plant material was collected in flowering and fruiting stage from kerle (Jaitapur). The collected plant specimen was identified by using taxonomic keys, flora of Kolhapur district &flora of Maharashtra [63]. The

preparation of herbarium of the collected plant specimen was deposited in the depositary of herbarium of Shivaji University, Kolhapur. The collected material was rinsed with water and then the material was shade dry. After shade dry material was put in the oven for 40 to 60ºc and completely dried material is crushed into the fine powder with the help of mechanical grinder. This fine powder was used for the further analysis. Bacterial strains obtained from Department of Microbiology, Shivaji University, Kolhapur those are *Staphylococcus aureus* (2654 NCIM), *Proteus vulgaris* (2813 NCIM), *Escherichia coli* (2832 NCIM), and *Bacillus cereus* (2703 NCIM).

1. **Extractive yield:** Extractive yield can be measured after the phytochemical extraction. Two gm of dry powder was used for the Soxhlet extraction and after extraction weight the solid material remaining in the thimble. For the calculation of extractive yield used the following formula.

$$\textbf{Extractive yield} = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = Weight of dry sample taken for the extraction
W_2 = weight of sample after extraction

2. **Fluorescence analysis:** Fluorescence behaviour study carried out by method of Chase and Pratt (1949) [[12]; Kokoski *et al*. (1958) [35]. The drug powder was treated separately with different chemical reagent and exposed to visible, short (254nm) and long wavelength (365nm). The chemicals like conc. H_2So_4, conc. HCL, Nitric acid, acetone, Ferric chloride, NaOH (aq. and alcoholic), KOH, and Iodine are used for study the fluorescence of plant part.
3. **Powder behaviour of extract:** Crude powder behaviour was studied by using different chemical reagents. The colour of the reagent and reaction mixture was gives the idea about the phytochemical which is present in plant sample. Powder behaviour was studied by chase and pratt (1949) standard method.
4. **Preliminary phytochemical analysis:** The collected sample were used for soxhlet extraction [17, 34, 38, 46]. Two gm of dry powder was put in the thimble and the thimble was placed in soxhlet apparatus for the extraction of phytochemicals.
   For the extraction solvent such as petroleum ether (30-50^{0}C), alcohol (60-70^{0}C), acetone (40-50^{0}C), chloroform (50-60^{0}C) and distilled water (80-100^{0}C) were used. These solvents show the presence of phytochemicals. After extraction solid extract found which is dissolve in particular solvent and used for the analysis. The phytochemical study was carried out for the observing the presence of these phytochemicals such as alkaloid, phenol, saponins, tannin, flavones, anthraquinones, carbohydrate, xanthoprotein, coumarin and glycosides, etc.
5. **Proximate analysis:** The proximate analysis study the different parameter such as Dry matter, moisture, crude fiber, crude fat, crude protein and ash content.
6. **Dry Matter and moisture:** Dry matter and moisture can be calculated by the standard method AOAC (1990) [7]. Two gm of plant sample was taken in reweighted evaporating dish which is put in oven at 105^{0}C for obtaining constant weight. Dry matter and moisture can be calculated by following formula:

$$\text{Dry matter} = \frac{\text{(Weight of dish+ weight of dried sample)- Weight of dish}}{\text{Weight of sample before drying}} \times 100$$

$$\text{Moisture content (\%)} = \frac{\text{(Weight of fresh sample- weight of dry sample)}}{\text{Weight of dry sample}} \times 100$$

Crude Fiber

Crude fiber content was determined by the following standard method of sadasivam and manikam (1992) [51]. Two gm of plant material was mixed with 200ml of H_2So_4 put on hot plate for 30 min. continuous heating with addition of bumping chips to maintain heating of sample. For removal of acid traces from sample washed with hot distilled water. After that again boiled with 200ml of NaOH for 30 min. and then washed with H_2So_4 then hot distilled water. Residue was again wash with 25 ml alcohol and then washed residue was put in the pre weighted crucible (W_1) and dried at 130±2^{0}c for 2 hours. Again taken the weight of that material after drying (W_2) and put in the muffle furnace to ignite at 30 min. for 600^{0}C. (W_3). The crude fiber was calculated by the formula.

$$\text{Crude fiber (\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \times 100$$

Total ash

Ash content was determined by using AOAC (1990) [7] standard method. Two gm of plant sample was put in the pre-weighted crucible and crucible was placed in muffle furnace at 600^0C for 6 hours. For cooling crucible was transferred in desiccator to avoid moisture. The percentage of total ash was calculated by following formula.

$$\text{Total ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude fat

The crude fat content was determined by sadasivam and manikam (1992) [51] method. Two gm of plant sample was taken in thimble and put in the Soxhlet for the extraction. For extraction petroleum ether solvent was used. After the complete extraction the solvent was pour in pre weighted evaporating dish (a) for the evaporation of excess ether. After evaporation cool the dish in desiccator and taken the weight (b). The following formula used for the calculation of crude fat.

$$\text{Crude fat}(\%) = \frac{(b\text{-}a)}{\text{Weight of sample}} \times 100$$

Crude Protein

Nitrogen content was estimated by the Hawk *et al.* (1948) [28] standard method. The crude protein was calculated by the multiplying the factor 6.25 to the nitrogen content (AOAC, 1990).

Mineral analysis

For the mineral analysis we can use the Kjeldahl's method for nitrogen and Sekine *et al.* (1965) [52] for phosphorus and for that acid digestion processes used. For the mineral analysis sample was mixed with proper solvent and then run in atomic absorption spectrophotometer. For the mineral analysis ash was prepared and dissolves in HCL run in atomic absorption spectrophotometer.

Antioxidant analysis

For Non-enzymatic and enzymatic antioxidant analysis fresh plant material was used.

Non-enzymatic antioxidant analysis

In the analysis of different parameter such as carotenoid, total polyphenol and ascorbic acid studied by kirk and Allen (1965) [33], Folin and Denis (1915) [19] and Sadasivam and Manikam (1992) [51] standard method was used respectively.

1. Carotenoid: The extract was prepared by adding 10 ml of 80% chilled acetone homogenised with 0.5 g of fresh plant sample and addition of pinch of $MgCO_3$ to neutralize acid which is released in extraction. The extract was filtered through Buchner funnel under suction by using Whatman no.1 filter paper. Final extract was made 100ml and which is wrap with black paper to avoid photo-oxidation and measure the absorbance of at 480 nm on UV-VIS double beam spectrophotometer, using 80% acetone as standard. Carotenoid was calculated by using following formula.

$$\text{Carotenoid} = \frac{A_{480} \times \text{volume of extract} \times 10 \times 100}{2500 \times \text{weight of plant material (g)}}$$

2. ii) Polyphenol: The extraction method was same as carotenoid. After the extraction the filtrate was used for the further analysis. From the extract take 2 ml sample which mixed with 10 ml of 20% Na_2CO_3 and make up to 35 ml final volume with distilled water. 2ml of folin- denis reagent mixed thoroughly in reaction assay and made final volume 50 ml with distilled water. Absorbance was recorded at 660 nm after 20 min. Standard polyphenol curve was prepared by using tannic acid. Following formula was used for total polyphenol calculation. Abs. of ml. of std Final volume

$$\text{Total polyphenol} = \frac{\text{Abs.of ml. of std. Final volume Plant extract} \times \text{taken for assay} \times \text{of extract}}{\text{Abs. of weight of plant Standard} \times 2 \times \text{material in g} \times 1000} \times 100$$

3. iii) Ascorbic acid: Fresh plant material of 0.5 g was crushed in 4% oxalic acid and final volume made to 100 ml with oxalic acid after centrifugation. The collected supernatant was titrating against dye (2, 6 dichloro phenol indophenol). Appearance of pink colour which is persisted for few min is the end point of reaction. Ascorbic acid is used as a standard. Ascorbic acid calculated by using the formula.

$$\text{Ascorbic acid} = \frac{0.5 \times \text{Burette reading (extract)} \times \text{Final volume of extract}}{\text{Burette reading (std.)} \times 15 \times \text{wt. of plant material in g}} \times 100$$

Enzymatic antioxidant analysis

In that analysis studied different parameter such as Catalase by Sadasivam and Manikam (1992) [51] and peroxidase by Maehly (1954) [39] standard methods. Extract for the analysis of catalase and peroxidase activity was prepared using the same method.

1. Catalase: Fresh plant material 0.5 g was homogenised in 10 ml 0.1 M potassium phosphate buffer maintain at pH 7. Filter through 4 layered muslin cloth and centrifuged at 10,000 rpm for 10 min at 0 to 4^0C. The extract was used for the further analysis. The enzyme assay contains 50µl of enzyme with 3 ml H_2O_2.The H_2O_2 was prepared by using potassium phosphate buffer. The optical density was measured per 30 sec. at 240 nm on UV- VIS double beam spectrophotometer. The activity calculated by using the following formula.

$$\text{Catalase} = \text{O.D. absorbance} \times \text{Std. protein abs.} \times \text{conc. Of std. protein} \times 2$$

2. Peroxidase: In the peroxidase assay reaction mixture contain 2ml of phosphate buffer, 1 ml of 20 mM guaiacol, 0.5 ml enzyme and after addition 0.1 ml of H_2O_2 reaction was initiated. for that analysis H_2O_2 was prepared in distilled water. Change in the optical density due to oxidation of guaiacol was recorded per 30 sec. at 470nm.Some of the modification in standard method was done according plant sample. The activity was expressed as O.D. $min^{-1}mg^{-1}$ protein.

The activity was calculated by the following formula.

$$\text{Peroxidase} = \frac{\text{O.D.abs.} \times \text{std. protein abs.} \times \text{conc.of std. protein} \times 2}{\text{Std.protein abs.} \times \text{ml.of std. taken for assay} \times \text{ml of enzyme taken for assay}}$$

Free radical scavenging activity:

In that radical scavenging activity studied different antioxidants which are as follows. for that analysis enzyme extract was prepared by addition of four different solvent such as methanol, ethanol, acetone and distilled water. 0.5 % enzyme source was prepared with the help of sonicator and after centrifugation directly used as enzyme source.

DPPH (1,1- Diphenyl-2- picryl- hydrazyl): The free radical scavenging activity was measured by Aquino *et al*. 2001 [8]. Plant extract 25 µl was mixed with 3 ml of 25mM DPPH methanolic solution. Reaction mixture was put in dark for 30 min. the absorbance was measured at 517 nm against blank. The activity was calculated using the following formula.

$$\text{DPPH inhibition (\%)} = \frac{\text{Control (abs)} - \text{Sample (abs)}}{\text{Control (abs)}} \times 100$$

FRAP (Ferric reducing antioxidant power)

The ferric ion reducing activity was calculated by using assay given by Pulido *et al.*2000 [48]. Plat extract 0.1 ml was mixed with 3 ml FRAP reagent (0.3M acetate buffer:

10mM TPTZ: 20 mM $fecl_3$ $^{[10:1:1]}$) assay was incubated at 37^0C for 15 min. Absorbance was recorded at 593 nm. The results were expressed as ascorbic acid equivalent antioxidant capacity. The samples were prepared in triplicate for each analysis.

TPC (total phenolic content)

Total phenolic content was measured by standard folin- ciocalteu method with slight modification described by wolfe *et al* 2003 [61]. The reaction assay contains 0.125µl of enzyme extract with 0.125µl folin- ciocalteu reagent and adds 1.25ml of saturated solution of Na_2Co_3. The mixture was incubated at 37^0C for 90 min. and measured the absorbance at 760 nm. Results were expressed as of gallic acid equivalent antioxidant capacity

SOAS (superoxide anion scavenging assay)

The reaction mixture contains 0.3 ml of enzyme, 0.1 ml of NBT (1mg in 1ml DMSO) and 1ml of alkaline DMSO (0.1 ml of 0.5mM NaOH in 0.9 ml of DMSO) was added and absorbance was measured at 560 nm. Decrease in value of absorbance of the reaction mixture designate the increase in superoxide anion scavenging activity (Tiwari *et al*., 2017) [56]. The SOAS activity was calculated by using formula:

$$\text{\% SOAS inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)}}{\text{Control (abs)}} \times 100$$

PMO (Phosphomolybdenum reducing power assay)

Plant extract 0.3 ml was combined with 3 ml of reagent (reagent contain 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95^{0}C for 90 min. the absorbance of the mixture was measured at 695 nm using UV – visible spectrophotometer against black. Methanol was used as a standard. Phosphomolybdenum method according to the procedure described by Prieto *et al*.(1999) [47]. Slight change in method according to plant.

$$\% \text{ PMO inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)}}{\text{Control (abs)}} \times 100$$

FICA (ferrous ion chelating activity)

The enzyme mixture contain 1 ml of plant extract mixed with 0.1 ml 2mM $fecl_2$ and 0.3 ml 5mM ferrozine. The mixture was incubated for 10min. at room temperature and absorbance was recorded at 562nm on UV – visible spectrophotometer. the ferrous ion chelating activity was measured by following method described by Dinis *et al*.1994 [15]. The ferrous ion was chelate by sample is calculated as the percent inhibition using the following formula.

$$\% \text{ Ferrous ion inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)}}{\text{Control (abs)}} \times 100$$

TFC (total flavonoid content): The total flavonoids were estimated by using modified colorimetric method (Luximon-Ramma *et al.*, 2002) [37]. The enzyme assay contain 1.5 ml plant extract mixed with 1.5 ml of 2% methanolic $AlCl_3$. The reaction mixture was incubated for 10 min. at room temperature and absorbance was measured at 368nm. The optical density measurements of samples were compared to standard curve of rutin as expressed as mg of rutin equivalent /g dry weight of plant part like leaves and stem bark.

Antimicrobial activity

Antimicrobial activity of plant extracts against bacteria like *S. aureus, P. vulgaris, E. coli, B. cereus* done by agar well diffusion method (Brown, D. 1995; Sharma *et al.,* 1998) [10, 16, 45]. A 100 µl of bacterial suspension according to Mcfarland CLSI, spread on sterile nutrient agar plate and wells (6mm) were made with the help of sterile cork borer. Plant extract concentrations were made and from each 100µl of respective plant extract were added into each well with triplicate manner. The plates were allow standing for 10 min.at 10^{0}C for diffusion, then incubated at 37^0 C for 24 hrs. The diameter of inhibition zone was measured after 24 hrs. of incubation for the test organisms. The inhibitory zones were measured and are compared with antibiotic such as streptomycin and ampicillin.

Result and Discussion

Preliminary phytochemical analysis was done from the various solvents like acetone, chloroform, Petroleum ether, ethanol and distilled water. The extractive yield for the leaves was shows that acetone extract was found to be more than other solvent extract while chloroform showing lowest extractive yield. In the stem bark, extractive yield shows highest yield in methanol while low in chloroform. The extractive yield of leaves was 8.33%, 8.44%, 6.44%, 9.34%, 7.88%, 5.33% in methanol, ethanol, petroleum ether, acetone, distilled water, chloroform, respectively. In present study stem bark shows the extractive yield was 10.21%, 9.24%, 6.33%, 9.44%, 7.35%, 6.01% in methanol, ethanol, petroleum ether, acetone, distilled water, chloroform, respectively.

In the fluorescence analysis used the different chemical reagents for the study of fluorescence of powder material of selected plant part. The fluorescence was measured by the fluorescence spectrophotometer which showing the different color in visible, short (254nm) and long (366nm) wavelength. In the analysis black colour was found frequently in both leaves and stem bark for the long wavelength (366nm) of light in all chemical reagents. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material [25]. Some of the substances may often converted into fluorescent derivatives by using different chemical reagents through they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation [41,44]. The visible and short wavelength (254 nm) for the leaves most of the time showing the greenish yellow or dark green color as well as stem bark was showing the dark brown color. The crude drug which is present in plant was observed by that analysis.

Powder behaviour of the plant was studied by using different chemical reagent. The powder shows presence of different color in daylight which indicating presence of various phytochemicals. In the powder behaviour study of leaves showing the presence of flavonoid, steroids, oil, xanthoprotein, glycosides and tannin. The stem bark powder behaviour was shows that flavonoid, tannin, steroide, lipid, xanthoprotein and glycosides like secondary metabolites which is helpful for the plant [49]. The result shows that presence of phytochemicals such as alkaloid, tannin, Saponin, xanthoprotein, carbohydrate, phenol, and flavones. The acetone and alcoholic extract showing presence of high number of phytochemicals as compare to other solvent systems. Saponins were showing their presence in all solvent with studied plant part. Herbs which contain tannin as main components are astringent in mature and are used for treating diarhea and dysentery (Dharmananda 2003) [14]. The presence of phytochemicals

has been recently considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular diseases, and diabetes [57]. Plant tannin was used for brewing industries in clarifying beer and wine. [30] Alkaloids are reported to have analgesic, anti-inflammatory and adatogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress. Tannins are known to inhibit LPO and lipoxygenase *in vitro*, and information has been accumulated over the last few years demonstrating their ability to scavenge radical such as hydroxyl, superoxide, and peroxyl, which are known to be important in cellular pro-oxidant state. Tannin are widely distributed and present in almost all plant foods and some beverages [59].

Saponins, a special class of glycosides, have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and antifungal properties [17, 53, 30]. In the proximate analysis it was shows that Dry matter(64.3%), crude fat(65.4%), crude fiber (40%)content was more in stem bark as compare to leaves and total ash(20%), moisture (40.5%)and crude protein (16.31%) was more in leaves. In the stem bark Ash, moisture, crude protein and in leaves crude fat, crude fiber, crude protein was found to be less.

Mineral analysis was shown in the table no.2. In the mineral analysis studied both the macro as well as micro elements. The macro elements such as nitrogen (2.61±0.02 gm/100gm) and phosphorus (0.35±0.03 gm/100gm), Potassium (1.32±0.1 gm/100gm), Calcium (0.76±0.01 gm/100gm) magnesium (0.47±0.4 gm/100gm) and Sodium (2.93±0.04 gm/100gm) was found in the leaves and Nitrogen(1.23±0.03gm/100gm), Phosphorus (0.23±0.05gm/100gm), Potassium(1.43±0.02 gm/100gm), Calcium (1.89±0.2 gm/100gm) Magnesium (0.52±0.1gm/100gm) and Sodium (2.24±0.003 gm/100gm) was observed in stem bark respectively. For the development of bone and regulation of heart beat and blood clotting, calcium is essential mineral. The mineral elements like Magnesium, sodium and potassium inorganic elements which involve in various biological processes in human and livestock [29]. As like macro elements, also studied the micro elements from the leaves and stem bark. The minerals such as Iron (0.020±0.04 mg/100gm), Manganese (0.012±0.03mg/100gm), Copper (8.707±0.2 mg/100gm) and Zinc (2.787±0.1 mg/100gm) was observed in stem bark and elements such as Zinc (21.571±0.08 mg/100gm), Iron (0.014±0.2 mg/100gm), Manganese (0.0006±0.06 mg/100gm), and Copper (6.557±0.05 mg/100gm) was in leaves. From the mineral analysis it was found that amount of macro and micro elements was higher in stem bark as compare to leaves in most of the minerals.

Antioxidant compounds may also regulate inflammatory and immune responses and may have antiulcerative properties. Antioxidant also acts as metal chelators and interferes with the pathways that regulate cell division, proliferation and detoxification [18, 60]. The use of various methods in antioxidant activity is necessary to assessment to understand the various antioxidant mechanisms which are operating in the cellular system was earlier suggested by the Frankel *et al.*1994 [20]. In the fresh plant antioxidant analysis studied both enzymatic and non-enzymatic antioxidant. Non enzymatic antioxidant interrupt free radical chain reaction while enzymatic antioxidant work by breaking down and removing free radicals [9]. In the enzymatic analysis activity of catalase (0.1203$Min^{-1}mg^{-1)}$ was highest in stem bark and peroxidase (0.7105$Min^{-1}mg^{-1}$) activity was found more in leaves respectively. The non-enzymatic analysis shows that carotenoid content 6.86mg/100g and 5.78mg/100g, polyphenol content 8.474mg/100g and 19.525mg/100g and ascorbic acid amount 10.8mg/100g and 20.8mg/100g was found in leaves and stem bark respectively. Polyphenol content and ascorbic acid was observed in stem bark which is double to the leaves. In the current work, 2,2 Diphenyl 1 picrylhydrazyl radical scavenging assay (DPPH), Superoxide anion scavenging assay (SOAS), Total phenolic content (TPC), Ferrous ion chelating activity (FICA), Phosphomolybdenum reducing power assay (PMO) and Ferric reducing antioxidant power assay (FRAP) assays were used to evaluate antioxidant properties of *Bridelia montana* leaves and stem bark extracts. DPPH is a free radical reacts an antioxidant, its free radical property is lost due to chain breakage and its color changes to light yellow. In the DPPH activity it was revealed that antioxidant scavenging activity was in range between 43.1% to 93%. From the selected solvent system ethanolic extract shows the maximum scavenging activity. Ethanolic stem bark extract was showing the high scavenging activity while methanolic stem bark extract showing the low scavenging activity. The DPPH scavenging activity of leaves was 68.36%, 83.6%, 77.17%, 46.5% in acetone, ethanol, methanol, and Distilled water extract respectively while 74.73%, 93%, 68.23% & 43.1% scavenging activity in acetone, ethanol, methanol and distilled water extract of stem bark respectively. In the ferric reducing antioxidant assay was study by the Pulido *et.al* standard method and the activity was measured in comparison with ascorbic acid equivalent. In that activity it was observed that acetone extract of leaves and distilled water extract of stem bark showing highest activity as compare to other solvent. In the leaves FRAP activity showing highest to lowest activity from acetone >distilled water>ethanol>methanol solvent respectively. In the stem bark FRAP activity showing lowest to highest activity from methanol<acetone <ethanol<distilled water respectively. In comparison of both leaves and stem bark, methanol extract showing highest activity in leaves and lowest activity in stem bark for this antioxidant activity. FRAP is primarily used for determining antioxidant activity of plasma, later successfully applied to measure the antioxidant activity of a number of biological sample and pure substances [20, 3]. The FRAP activity was showing range between 49.37 mg to 643.09 mg/100g. In the FRAP antioxidant activity acetone extract of leaves showing maximum activity while methanolic extract of stem bark sowing the minimum activity as compare to other solvent system. Phenolic compounds are having the antioxidant properties mainly because of their redox potential, which allow them to act as reducing agent, hydrogen donors, singlet oxygen quenchers and metal chelators [6]. In addition, plant phenolic compounds have also been found to be good metal ion chelators [27]. The aqueous extract had the

highest phenolic content followed by ethyl acetate and ethanolic extract of *Bridelia ferruginea* was reported by olaide olarewaju *et.al.*2014 [43]. For *B.montana* the acetone, methanol, ethanol and distilled water extract of leaves shows significant amount of total phenol content (TPC) with the value of 229.10 mg, 2380.53 mg, 1538.35 mg, and 427.03 mg/100g, respectively. The acetone, methanol, ethanol, distilled water stem bark extract significant amount of TPC with the value of 137.77 mg, 2141.36 mg, 1366.38 mg and 333.48 mg/100g, respectively. In these studies it was observed that both ethanolic and methanolic extract showing the maximum activity as compare to acetone and distilled water extract. In leaves highest activity was found in ethanol (1538.35mg/100g) and in stem bark highest activity was found in methanol extract. For both leaves as well as stem bark showing minimum activity in acetone extract. The antioxidant activities of TFC showing exactly opposite result to TPC. In total flavonoid content activity it was observed that methanolic extract of leaves and ethanolic extract of stem bark showing the maximum activity which is exactly opposite to activity of TPC. Flavonoids as important component in human diet but never considered as nutrient [9]. Many flavonoids may help to provide protection against the oxidation at the cellular level as antioxidants by interfering in enzyme activity, chelating of redox active metals and effective scavengers of hydroxyl and peroxyl radicals as well as quenching superoxide radicals and singlet oxygen [20]. The polyphenolic compounds like flavonoids possessing the majority of plant secondary metabolites and have shown to possess remarkable health promoting effects including antioxidant activity [50, 57]. The activity was showed their presence in range between 24.12 mg to 976.16 mg/100g. The acetone extract of both leaves and stem bark showing lowest activity in comparison with ethanol, methanol, and distilled water extract. The maximum activity was observed in methanol extract of leaves (976.16 mg) and ethanol extract of stem bark (697.34 mg/100g). The activity was measured against the rutin equivalent. The Phosphomolybdenum (PMO) antioxidant activity of plant was measured by the percentage inhibition method. Anjum *et al.*2013 earlier studied three different *Bridelia* species which is *Bridelia tomentosa, Bridelia stipularis, Bridelia verrucosa*. In that study they observed that aqueous extract of leaves of *B.stipularis* shows the highest amount of phenolic content and methanolic extract of stem bark contain lowest amount of phenolic content [5]. Total antioxidant capacity of extract was determined by phosphomolybdenum assay where the highest value was found in methanolic extract of leaf of B. tomentosa followed by methanolic extract of bark and leaf of B. verrucosa as evident from 612.54mg, 542.88mg, and 484.43mg equivalents of ascorbic acid respectively [43]. In the leaves and stem bark PMO activity was observed highest in methanolic extract while lowest in acetone extract. The methanol extract of stem bark showing highest activity while acetone extract of stem bark showing the minimum activity. The PMO activity in leaves and stem bark was observed in between the range of 9.10 mg/100g to 77.18 mg/100g. In the ferrous ion chelating activity of *B. montana* it was observed that both highest and lowest activity in stem bark. The ethanol extracts for leaves (73.31%) and stem bark (78.96%) showing the maximum antioxidant activity while methanol extracts of leaves (14.94%) and acetone extract of stem bark (2.18%) showing the minimum activity. In the leaves ferrous ion chelating activity showing highest to lowest activity from ethanol > acetone > distilled water > methanol respectively. In the stem bark FICA activity was showing highest to lowest activity in ethanol > methanol > distilled water > acetone respectively. For SOAS (superoxide anion scavenging activity) activity of leaves and stem bark highest activity was observed in ethanol extracts and lowest activity in acetone extracts. The superoxide anion scavenging activity of leaves was 10.87%, 60.49%, 54.77%, and 47.53% in acetone, ethanol, methanol, and distilled water extract respectively. The SOAS activity of stem bark was 29.03%, 74.98%, 36.58%, and 37.8% in acetone, ethanol, methanol, and distilled water extract respectively. In the studied antioxidant analysis it was clearly observed that PMO, TPC, TFC, DPPH, SOAS, and FICA were showing maximum activity in ethanol and methanolic extracts while FRAP showing near about similar activity in acetone, ethanol, and distilled water extracts. The distilled water and acetone are showing lowest activity of the antioxidant. From that it was observed that ethanolic and methanolic extracts are more suitable for antioxidant activity of leaves and stem bark of *B.montana*. Antimicrobial activity of the plant was studied by the agar well diffusion method. The methanolic extract of *Bridelia montana* leaves and stem bark were prepared. In that study of antimicrobial activity it was observed that leaves extract of plant was showing the highest zone of inhibition as compare to stem bark. The streptomycin and ampicillin was used as standard for the study of activity of bacteria. The plant extract was showing the maximum zone of inhibition as compare to standard. The extract of leaves and stem bark showing the maximum zone of inhibition for *Staphylococcus aureus* and minimum zone of inhibition for *Proteus vulgaris*. When extract Concentration increases, zone of inhibition also increases that. In present study gram positive bacteria are more sensitive to plant extract as compare to gram negative bacteria.

Table 1: fluorescence study of leaves and stem bark of *Bridelia montana*.

Sr. No.	Partsused	Treatment	wavelength		
			Visible	Short (254nm)	Long (366nm)
1.	Leaf	Powder	Green	Pale green	Dark black
		Powder+ distilled water	Green	Dark green	Dark black
		Powder + NaOH (aq.)	Greenish yellow	Yellowish black	Black
		Powder + NaOH (alco.)	Purple	Dark green	Black
		Powder + acetone	Green	Dark green	Black
		Powder+ conc.HCL	Green	Yellowish green	Black

		Powder + conc.H2So4	Greenish yellow	Dark green	Black
		Powder + 10%HCL	Green	Dark green	Dark black
		Powder + 5%KOH	Yellowish green	Greenish	Black
		Powder +5% fecl3	Green	Dark green	Black
		Powder + conc.HNO3	Yellowish green	Yellowish green	Black
		Powder + iodine	Green	Green	Dark green
2.	Stem bark	Powder	Brown	Yellowish brown	Black
		Powder+ distilled water	Brown	Dark brown	Black
		Powder + NaOH (aq.)	Chocolate brown	Blackish brown	Black
		Powder + NaOH (alco.)	Chocolate brown	Dark brown	Black
		Powder + acetone	Brown	Dark brown	Black
		Powder+ conc.HCL	Brown	Copper brown	Black
		Powder + conc.H2So4	Black	Coffee brown	Black
		Powder + 10%HCL	Brown	Yellowish brown	Black
		Powder + 5%KOH	Coffee	Chocolate brown	Black
		Powder +5% fecl3	Brown	Greenish black	Black
		Powder + conc.HNO3	Yellow brown	Olive green	Black
		Powder + iodine	Brown	Pale green	Black

Table 2: Extractive yield of leaves and stem bark of *B. montana*

Sr. No.	Solvent system	Plant material	
		Leaves (%)	Stem bark (%)
1	Methanol	8.33	10.21
2	Ethanol	8.44	9.24
3	Petroleum ether	6.44	6.33
4	Acetone	9.34	9.44
5	Distilled water	7.88	7.35
6.	Chloroform	5.33	6.01

Table 3: Powder behaviour study of *Bridelia montana* leaves and stem bark

Sr. No.	Part used	Treatment	Behaviour	Inference
1.	Leaf	Powder as such	Pear green	---------
		Powder+ 1N NaOH	Greenish yellow	------
		Powder + 5% Iodine	Yellow green	-
		Powder + 40% NaOH + lead acetate	Verde bottiglia green	---------
		Powder + Glacial acetic acid	Green	-------
		Powder + Conc. H_2So_4	Brown	Steroids
		Powder+ 5% $FeCl_3$	Dark green	Flavonoid, tannin
		Powder + 5% KOH	Dark brown	Glycoside
		Powder + Conc.HNO_3 + Ammonia	Orange	Xanthoprotein
		Powder + Picric acid	Caramel brown	------
		Powder + Sudan III	Wine red	Oil
2.	Stem Bark	Powder as such	Sandstone	-----
		Powder+ 1N NaOH	Light brown	Flavonoid
		Powder + 5% Iodine	Brown	-
		Powder + 40% NaOH + lead acetate	Light brown	------
		Powder + Glacial acetic acid	Rusty orange	----
		Powder + Conc. H_2So_4	Dark brown	Steroids
		Powder+ 5% $FeCl_3$	Olive green	Flavonoid, tannin
		Powder + 5% KOH	Brown	Glycoside
		Powder + Conc.HNO_3 + Ammonia	Rusty orange	Xanthoprotein
		Powder + Picric acid	Yellow orange	------
		Powder + Sudan III	Orange	---

Table 4: Preliminary phytochemical analysis of *Bridelia montana* Leaves and stem bark

Sr. No.	Solvent	Leaves					Stem bark				
		Alc.	Ace.	Pet. ether	Dw	Chl.	Alc.	Ace.	Pet. ether	Dw	Chl.
1	Alkaloid	+	+	-	-	-	+	+	-	-	-
2	Phenol	+	+	+	-	+	-	+	-	-	-
3	Tannin	+	-	+	+	+	-	+	+	+	+

4	Saponin	+	+	+	+	+	+	+	+	+	+
5	Carbohydrate	+	-	-	+	-	-	+	-	+	-
6	Xanthoprotein	+	+	-	-	-	-	-	-	+	-
7	Flavones	+	+	-	+	-	+	+	-	+	-
8	Anthraquinone	-	-	-	-	-	-	-	-	-	-
9	Caumarin	-	-	-	-	+	-	-	-	-	-
10	Glycosides	-	-	+	-	+	-	-	+	-	-

Table 5: Major element analysis of leaves and stem bark of *Bridelia montana*

Sr. No	Minerals	Leaves	Stem bark
1	Nitrogen (g/100gm)	2.61±0.02	1.23±0.03
2	Phosphorus (g/100gm)	0.35±0.03	0.23±0.05
3	Potassium (g/100gm)	1.32±0.1	1.43±0.02
4	Calcium (g/100gm)	0.76±0.01	1.89±0.2
5	Magnesium (g/100gm)	0.47±0.4	0.52±0.1
6	Sodium (g/100gm)	2.93±0.04	2.24±0.003

Table 6: Minor element analysis of leaves and stem bark of *Bridelia montana*

Sr. No	Minerals	Leaves	Stem bark
1	Iron (mg/100gm)	0.014±0.2	0.020±0.04
2	Manganese (mg/100gm)	0.0006±0.06	0.012±0.03
3	Zinc (mg/100gm)	21.571±0.08	2.787±0.1
4	Copper (mg/100gm)	6.557±0.05	8.707±0.2

Table 7: Antimicrobial activity of *Bridelia montana* leaves and stem bark extract

Sr.no.	Organism	Conc.	Leaves	Stem bark
1.	*Staphylococcus aureus* (gram + ve bacteria)	Control	3.4	2.9
		25%	2.53	1.06
		50%	2.53	1.13
		75%	2.6	1.16
2.	*Bacillus cereus* (gram + ve bacteria)	Control	1.5	1.5
		25%	2.8	1.26
		50%	2.96	1.36
		75%	3.16	1.56
3.	*Proteus vulgari* (gram - ve bacteria)	Control	1.5	1.5
		25%	1.46	0.8
		50%	1.53	1
		75%	1.66	1.26
4.	*Escherichia coli* (gram - ve bacteria)	Control	1.6	1.6
		25%	1.46	1.1
		50%	1.7	1.3
		75%	1.7	1.43

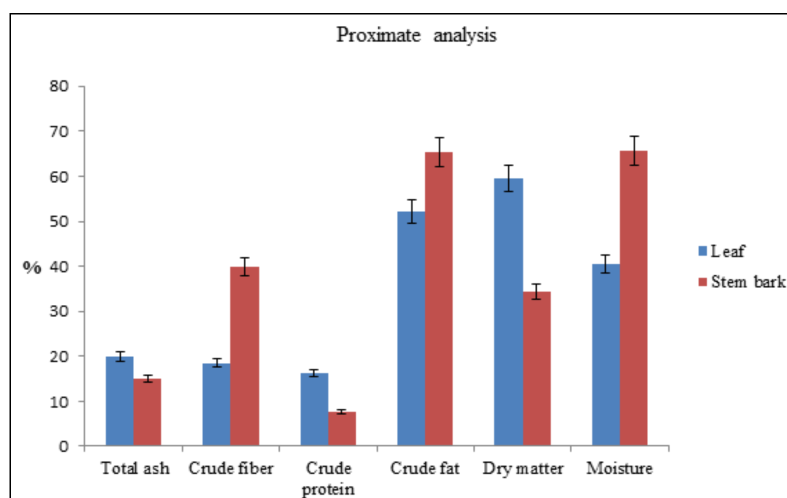

Fig 1: Graphical representation of Proximate analysis of *Bridelia montana*

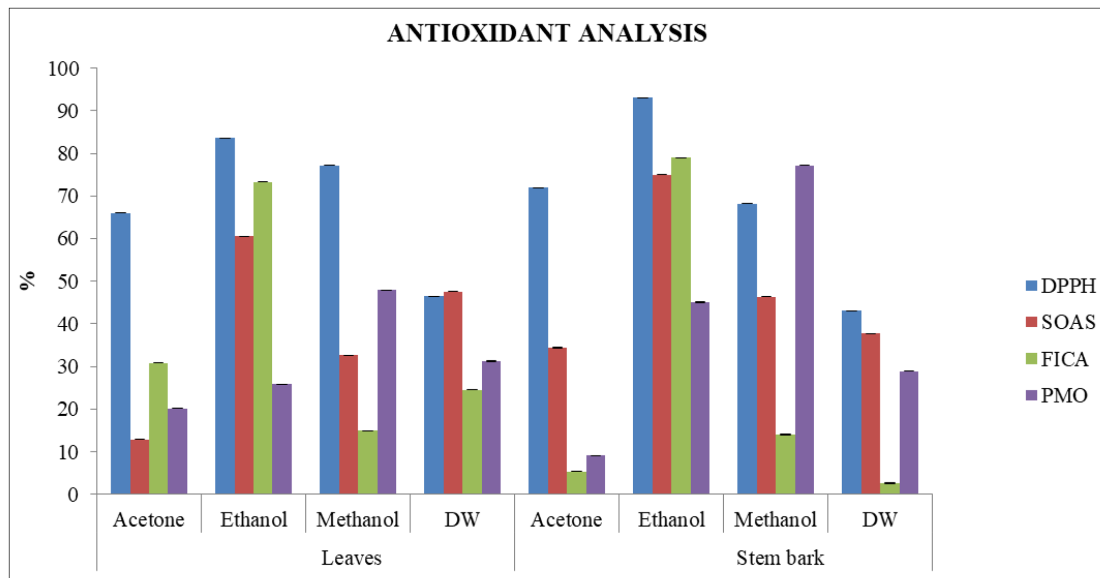

Fig 2: Graphical representation of Antioxidant analysis of *Bridelia montana*

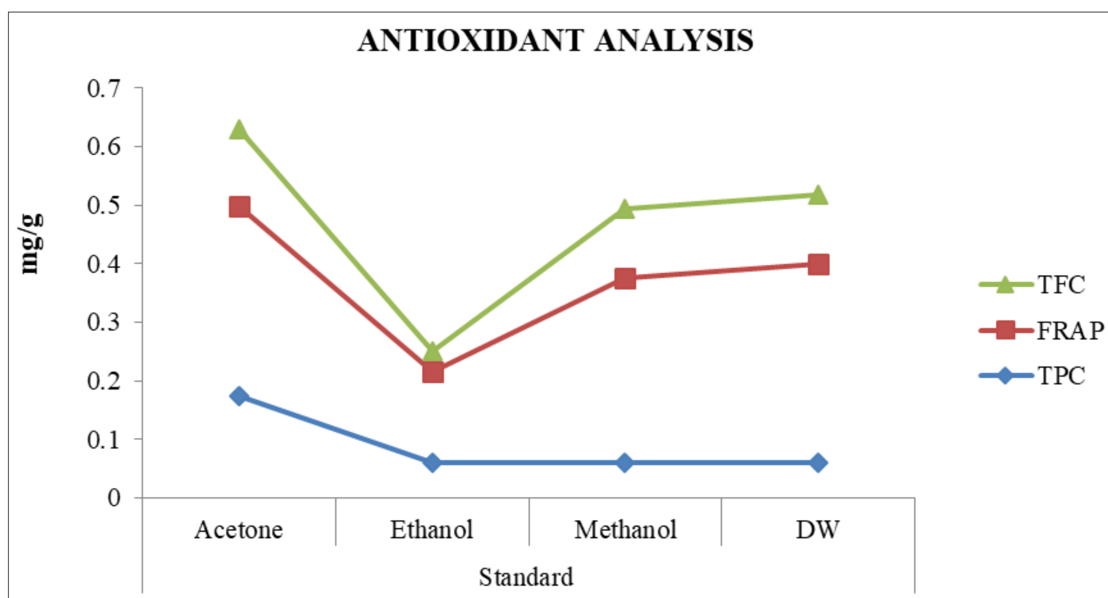

Fig 3: Graphical representation of Standard used for Antioxidant analysis

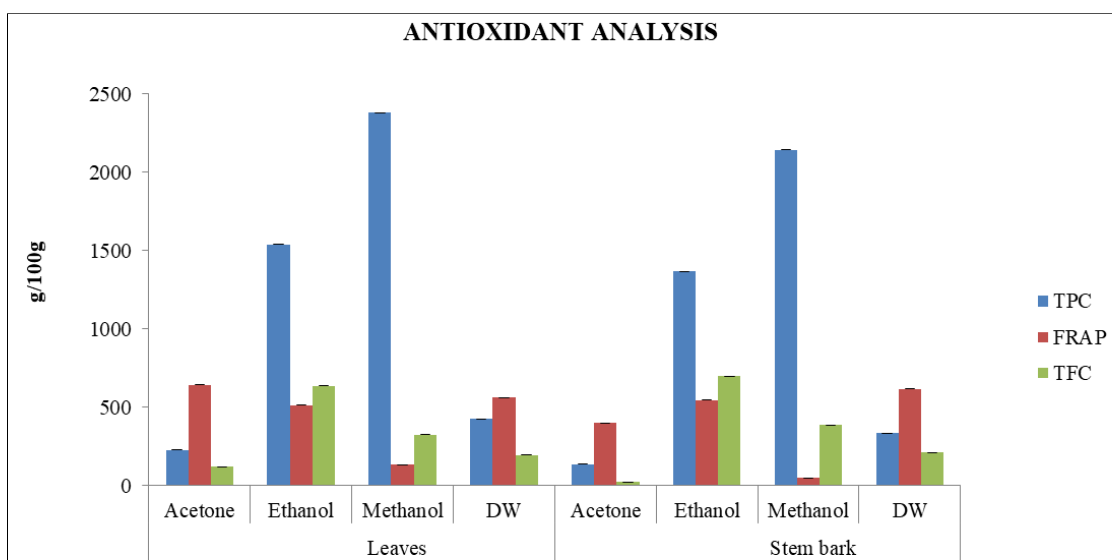

Fig 4: Graphical representation of Antioxidant analysis of *Bridelia montana*

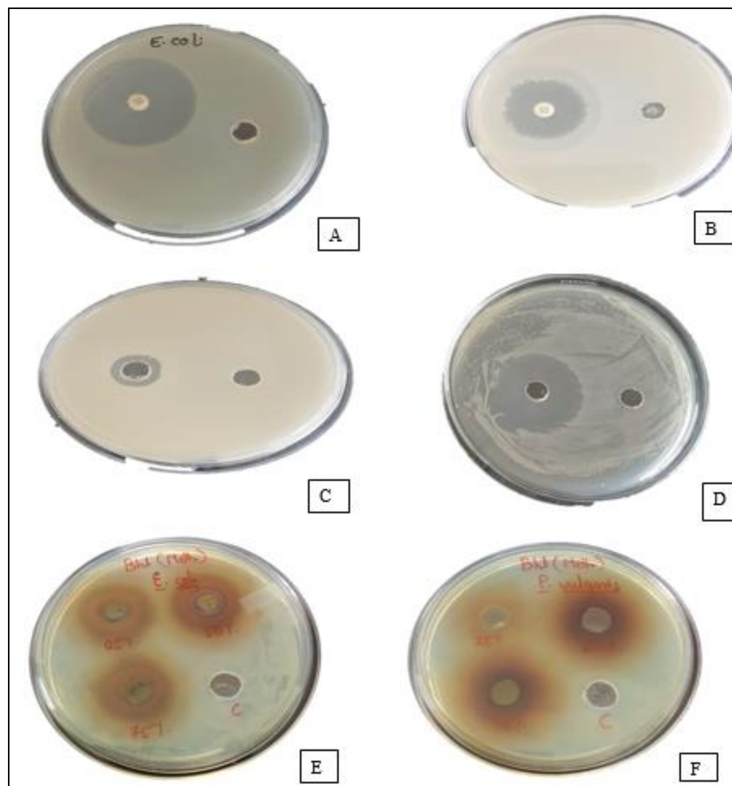

Fig 5: A, B, C, D – Showing the standard ampicillin with E. coli, *Staphylococcus aureus*, *Bacillus cereus, Proteus vulgaris,* respectively. Fig no E, F – showing the *Bridelia montana* leaf extract activity on different bacteria.

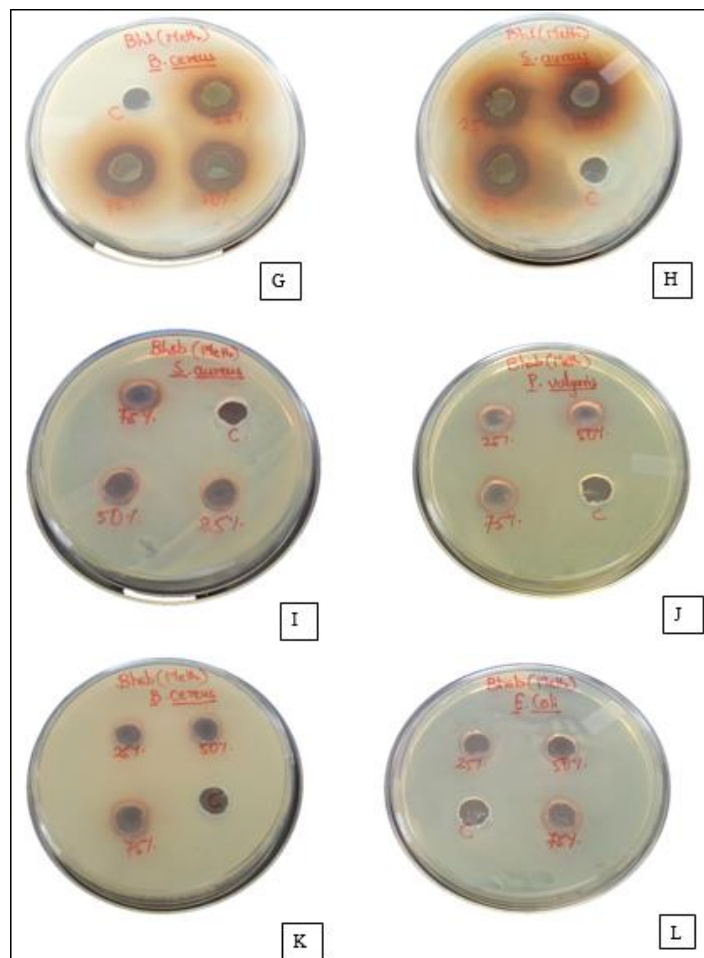

Fig 6: G, H – showing the *Bridelia montana* leaf extract activity on different bacteria.
Fig no I, J, K, L – showing the *Bridelia montana* stem bark extract activity on different bacteria. acteriaacterial.

Conclusion

Primary phytochemical analysis is important for analysing nature of crude drug. Alcohol, acetone and distilled water are the effective solvent for the extraction of phytochemicals from the plant extract. Phytochemicals which is present in plant showing the different activities like antioxidant, anti-inflammatory, antifungal, antimicrobial and antidiabetic activities. The ash content gives idea about the minerals present in plant. *Bridelia montana* shows the maximum amount of minerals. In present study, Crude protein content was high in leaves which indicate highest nutritional potential of leaves. For the determination of quality and purity of drug, fluorescence analysis of powdered drug is play important role. In this study, result revealed that plant extract possess potential antioxidant and antimicrobial activity. The results of present study supports the traditional usage of the studied plant and suggests that plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Leaves extract having maximum antimicrobial activity which is due to secondary metabolites. In present antioxidant study of plant extract shows that ethanolic extract are more suitable for showing the antioxidant activity. The DPPH, SOAS, FICA and TFC highest activity in ethanolic extract, TPC and PMO high in methanolic extract and FRAP activity was found high in acetone extract for both leaves and stem bark material. The active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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