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Studies on Chemical and Biological properties of 
Bryonia epigaea (Rottler)

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To investigate the phytochemical, antimicrobial and antioxidant potentials of 
Bryonia epigaea (Rottler). The study was performed by using various in-vitro methods such as 1, 1 Diphenyl 2 picryl hydrazyl (DPPH), and Agar well diffusion method for different concentrations of methanolic extracts. Phytochemical constituents of the three extracts (Hexane, Acetone and Methanol) were determined. Total Phenol content was determined by Folin Ciocalteu Method. Yield of extract was determined by calculating the mass of plant material before minusing the mass of the plant material after the extraction process. Extracts of Bryonia epigaea (Rottler) contained saponins, flavonoids, steroids, tannins, alkaloids, coumarins, phenols and reducing sugars. The antimicrobial activity was moderate. Methanol extract showed good antibacterial activity at 100 mg/ml concentration while antifungal activity against four significant pathogens was moderate. Bryonia epigaea (Rottler) methanol extract exerted significant antioxidant activity and dose dependent effect. The Results showed that the methanol extract showed high amount of antioxidant compounds and exhibit significant antioxidant activity. The antioxidant activity was found to be increased with the concentration of the compound. In the present research work we tried to find out the bioactive properties of ancient medicinal plant Bryonia epigaea (Rottler). The phytochemical, antimicrobial and antioxidant properties have been discussed.

Key words: Bryonia epigaea (Rottler), phytochemical analysis, antimicrobial properties, antioxidant properties.

INTRODUCTION

Plants are the most reliable sources of phytochemical components in the world and have efficient and economically useful chemical compounds that are to be isolated and characterised. There is a dreadfull need for the human world to eradicate the much threat full diseases by utilising the natural components (Nostro

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et al., 2000). *Bryonia*, a genus of perennial flowering and climbing plant belonging to the family Cucurbitaceae is most diversified with approximately 125 genera and 960 species. The Cucurbitaceae family is having pretty full of medicinal properties and is also having utilitarian chemical components. *Bryonia epigaea* (Rottler.) International plant name index (IPNI) is a perennial, tendril climber with a large, turnip shaped root and succulent commonly known as Muru Donda, Domma Donda, Naga Donda in Telugu and Garuda gaddah, Akasagaddah in Hindi (Kirtikar and Basu 1996). *Bryonia epigaea* (Rottler) is also called as *Corallo carpus epigus* and it is having the rhizome as a root. The root has Anti-diabetic (Kattamanchi et al., 2013), Anthelmintic activity (Shri Vijaya et al., 2011), Analgesic, Antipyretic and Anti-Inflammatory (Narendra et al., 2012). Anti-Arthritis (Patel et al., 2012) and Anti venom activities (Chandrakala et al., 2013). Leaves are having hepato protective activity (Rangu Mahesh et al., 2012). *Bryonia epigaea* (Rottler) is a well-known name in traditional medicine and folklore medicine. We tried to work with whole aerial parts of the plant and screened for Phyto active compounds, Antimicrobial activity and Anti-oxidant activity. The plant root has been used for snake bite in the tribal areas of Kurnool district and the Sriharikota island, Andhra Pradesh, India (Khaleel and Sudarsanam, 2012), (Bharath and Suryanarayana, 2011), the tribes of seshachalam forest use fresh roots in treating diabetes (Pavani et al., 2012). In Maruthamali hills of south Western Ghats of Tamilnadu dried roots are used for treating joint pains (Sarvalingam et al., 2011) and in the Salem region of Tamilnadu root powder is used for treating poisonous animal bites (Thirunarayanan, 2013). In tribes of Yavatmal District, Maharashtra, India: root powder is used for anorexia and snake bite (Borkar et al., 2012). The tribes of Buldhana Dist, Maharashtra, India use the decoction of the tuber in treating typhoid fever and its paste is used for treating swellings and poisonous strings. (Korpenwar, 2012). The tribes of Hoshangabad district, Madhya Pradesh, India use root powder decoction for treating chronic mucous entritis and dysentery (Manish and Upadhyay, 2011). In folklorie medicine of Meena community of Rajasthan, India, the root is used to cure stomach tumour, typhoid and diarrhoea. (Ajay and Rao, 2010). On Eastern Ghats of Peninsular India the root is a valuable remedy for rheumatism and also used in treating dysentery. Root paste is also a remedy for snakebites. (Sri Rama et al., 2013).

**MATERIALS AND METHODS**

**Plant collection**

Plant material was collected from the Nalamala Forest of Chittore district of Andhra Pradesh in the month of January to March. It was identified based on its floral characters, herbarium and including other pertinent taxonomic literature by Dr. S. M. Khasim, Botanist and Assistant Professor, Department of Botany, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India. It was shade dried and pulverized by the use of a grinder and stored in bags at room temperature.

**Collection of microorganisms**

The micro organisms used for the experiments were procured from MTCC, IMTECH, Chandighar and they were reconfirmed by gram staining, sub culturing in appropriate selective media and biochemical tests.

**Gram-positive organisms**

*Staphylococcus aureus* (MTCC 3160), *Streptococcus mutans* (MTCC497), *Lactobacillus casei* (MTCC1423), *Lactobacillus acidophilus* (MTCC495) and *Bacillus megaterium* (NCIM2187).

**Gram-negative organisms**

*Enterococcus faecalis* (MTCC439), *Xanthomonas campestris* (MTCC2286), *Escherichia coli* (ATCC35218) and *Pseudomonas aeruginosa* (ATCC 9027).

**Fungal strains**

*Candida albicans* (ATCC10231), *Aspergillus niger* (ATCC1015), *Rhizopus oryzae* (MTCC262) and *Candida rugosa*.

**Extraction procedure**

The plant material was extracted by using the Soxhlet Extraction Apparatus. *Bryonia epigaea* (Rottler) was extracted with three solvents hexane, acetone and methanol. The soxhlet extraction method is the most appropriate process to obtain the crude extracts and it was dependent on the boiling point of the solvents. The powdered plant material was weighed (50 g) and packed in the thimble. It was extracted with one litre of appropriate solvent (Hexane, Acetone, Methanol) and it was performed up to 30 to 40 cycles or till the solvent colour changes to orange red. The solvent in the round bottom flask was collected for the condensation with the rotary evaporator to minimise the solvent wastage and to concentrate the extract for the further usage. The crude extract was obtained from the rotary evaporator that it can be condensed in the vacuum at 50°C. The Yield of extract was determined by the calculation of weight before and after the extraction process.

**Phytochemical analysis**

This is according to Evans (1989), Gokhale et al. (1993), Trease and Evans (1996) and Harborne (1998). The phyto active compounds are analysed by conducting qualitative tests. The extracts are screened for alkaloids, steroidal compounds, flavonoids, saponins, phenolic compounds, tannins, coumarins and cardiac glycosides by using standard procedures.
Detection of alkaloids

Extract + Dil HCl + filtration.

**Mayer’s test:** Filtrate + Mayer’s reagent (Potassium Mercuric Iodide) = yellow colour precipitate.

**Wagner’s test:** Filtrate + Wagner’s reagent (Iodine in Potassium Iodide) = brown/reddish precipitate.

**Dragendroff’s test:** Filtrate + Dragendroff’s reagent (solution of Potassium Bismuth Iodide) = Red precipitate.

**Hager’s test:** Filtrate + Hager’s reagent (saturated picric acid solution) = yellow colour precipitate.

Detection of phenols

**Ferric chloride test:** Filtrate + freshly prepared 1% Ferric Chloride and potassium ferrocyanide = bluish-green colour.

**Ferric sulphate:** Filtrate + ferric sulphate = dark-violet colour.

Detection of flavonoids

**Alkaline reagent test:** Filtrate + NaOH solution = yellow colour + Dil HCl acid = Colourless.

**Lead acetate test:** Filtrate + lead acetate solution = yellow colour precipitate.

Detection of anthraquinones

**Free anthraquinones test:** (Borntrager’s test): Filtrate + 10 ml of benzene + filtered + 5 ml of 10% ammonia solution = pink, red, or violet colour in the ammonia (lower) phase.

**Modified borntrager’s test:** Filtrate + Ferric Chloride soln + water bath + extracted with benzene + ammonia solution = rose-pink colour in the ammonial layer.

Detection of phytosterols

**Salkowski’s test:** Filtrate + 2 ml chloroform + Conc. Sulfuric acid = A reddish brown colour at the interface.

**Libermann Burchard’s test:** Filtrate + chloroform + acetic anhydride + Conc. Sulphuric acid = brown ring at the junction.

Detection of terpenoids

Filtrate + acetic anhydride + conc H₂SO₄ = blue, green rings.

Detection of fatty acids

Filtrate + 5 ml of ether + evaporation on filter paper = transparence on filter paper (Rangu et al., 2012).

Detection of tannins

**Ferric chloride test:** Filtrate + 10% ferric chloride solution = bluish black.

**Lead acetate test:** Filtrate + 10% Lead acetate solution = yellow precipitate.

**Pot. dichromate test:** Filtrate + strong potassium dichromate solution = yellow colour precipitate.

Detection of saponins

**Froth test:** Filtrate + shaken in a graduated cylinder for 15 min = 1 cm layer of “honey comb” froth.

**Anthocyanins:** Filtrate + 2 ml of 2N HCl and ammonia = pink-red turns blue-violet (Shri et al., 2011).

**Leucoanthocyanins:** Filtrate + 5 ml of isoamyl alcohol = Upper layer appears red in colour (Shri et al., 2011).

**Coumarins:** Filtrate + 10% NaOH = yellow colour (Skerget et al., 2005).

**Emodins:** Filtrate + 2 ml of NH₄ OH and 3 ml of Benzene = red colour (Skerget et al., 2005).

Detection of reducing sugars

**Fehling’s test:** Filtrate + dil. HCl + alkali + heated with Fehling’s A & B solution = red precipitate.

**Keller - Kiliani test (for de-Oxy sugars in cardiac glycosides):** Filtrate + 2 ml chloroform + conc H₂SO₄ = Brown ring at interphase.

Antimicrobial activity

**Agar well diffusion method**

The Antimicrobial sensitivity test was performed *in vitro* to find out the efficiency of the extract that can resist the growth of the microorganism. Nutrient broth (NB) was prepared and inoculated with the respective microorganism. They were incubated for 24 h with constant shaking by orbital shaker. The nutrient agar (NA) plates were cooled to above room temperature and to these plates 10 µl of cultured nutrient broth was added. Four wells of 10 mm diameter were prepared using a cork borer. 50 µl of plant extract at a concentration of 10 mg/ml were added to the each well by using the sterile micro pipette and they are allowed to diffuse at room temperature for 2 h. These plates were incubated at 37°C for 18 to 24 h. Antibiotic sensitivity was also studied with different concentrations of the extracts to find out their effective dosage response. Sabouraud Dextrose Agar (SDA) plates were swabbed (sterile cotton swabs) with 24 h old - broth culture of respective fungi. Four wells (10 mm diameter) were made in each of these plates using sterile cork borer. About 50 µl of different concentrations (25, 50, 75 and 100 mg/ml) of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 28°C/72 h for the growth of fungal pathogens. The respective
extracts were maintained and solvents were used as a control. The experiment was repeated thrice, and average values of zone of inhibition were recorded in mm for analyzing the antimicrobial activity. The antibiotic compound Streptomycin (10 mg/ml) was used as a Standard for the antibacterial study (Perez et al., 1990).

**Determination of total phenols**

**Folin Ciocalteu method**

The total phenol contents were determined by the Folin Ciocalteu procedure by Skerget et al., (2005). Briefly, 1 ml of different concentrations of the Methanol extracts were taken and to that 0.1 ml of Folin Ciocalteu reagent and 2.5 ml of 0.2 N Na₂CO₃ was added and incubated for 30 min at room temperature. Distilled water was used as a reference blank. Absorbance was measured at 760 nm using Thermo Fisher double beam spectrophotometer. Gallic acid was used as standard and the results were expressed as mg of Gallic acid equivalents per gram dry mass of the extract (mg GAE gDM).

**Antioxidant properties**

1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) scavenging activity: The free radical scavenging activity of the extract was measured by using 1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) as described by (Brand-Williams et al., 1995) with some modifications. The methanol crude extracts were prepared at 1 mg/ml concentration with DMSO solution. The mixture was made uniform and working solutions were prepared at different concentrations. The 0.004% (W/V) solution of DPPH in methanol was added to the solution. The mixtures were shaken and incubated for 60 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank (Distilled water). The DPPH scavenging activity (I%) was calculated as follows:

\[
I\% = \frac{(A_o - A_s)}{A_o} \times 100
\]

Where Ao, is the absorbance of the DPPH solution without sample extract and As, is the absorbance of sample with DPPH solution.

**RESULTS AND DISCUSSION**

The yield of the extract was measured and it was 21.048% of the methanol extract, the colour of the extract was dark green in colour. The other two extracts had less yield and hexane appeared dark brown in colour (Figure 1). The yield of extract was more for the high polar solvents as most of the bioactive constituents are dissolved in high polar organic solvents. The qualitative analysis is a preliminary study for detecting the new compounds having biological significance. A general analysis has done to characterise the chemical nature of Bryonia epigaea (Rottler.). Phytochemical analysis of the three extracts (hexane, acetone, methanol) have successive amounts of chemical constituents. Hexane extract was found to have more compounds like flavonoids, saponins and coumarines when compared with acetone and methanol extracts. Saponins are used in the medicine as an antioxidant, anti-inflammatory, anticancerous agent and for hyperglycaemia, weight loss and it is also a strong pro active agent of the plant. Saponins also contain antifungal property (De-lucca et al., 2005). Acetone extract contained alkaloids, flavonoids, steroids, tannins, saponins, coumarins and reducing sugars. The methanol extract showed the presence of alkaloids, phenolics, steroids, saponins, tannins and reducing sugars (Table 1). Plants are the major reservoirs of Alkaloids and Flavonoids are more admirable for their curative effect of various allergies and carcinogens. They also show anti-cancerous, anti-microbial and anti-inflammatory activities (Harborne, 1973; Aiyelaagbe and Osamudiamen, 2009). The antimicrobial efficiency of Bryonia epigaea (Rottler) aerial part extracts showed good results against various pathogens (Figure 2).

It was found that all the three extracts have significant activity against the nine pathogens and Pseudomonas aeruginosa was remained unreactive for all the extracts. The activity was not affected by the concentration of the extracts. Methanol extract has more number of phyto constituents and has higher phenol content. Based on these results methanol extract was subjected to study by taking four concentrations and it was shown that 100 mg/ml concentration of methanol extract showed significant activity against all the microorganisms and Pseudomonas aeruginosa remained ineffective to all concentrations. The lower concentrations showed almost similar inhibitory activity (Figure 3). Anti-fungal activity was found to be moderate against all four concentrations of methanol extracts (Figure 4). The total phenol content of methanol extract was 327.68 µg GAE/500 µg (Figure 5). The DPPH radical scavenging activity was due to the hydrogen donating ability of the reagent and more over a substance when it is mixed with the DPPH solution, it can donate a hydrogen atom converted into the reduced form of 1,1-diphenyl-2-picryl hydrazine non radical form (Molyneux, 2004). The antioxidant property of Bryonia epigaea (Rottler) was evaluated by using the 1, 1-diphenyl 2-picryl (DPPH) method of radical scavenging activity.

The concentrations of 100 µg/ml to 500 µg/ml showed a gradual increase in the percentage of scavenging activity of four extracts shown in Figure 6. The values were shown along with the positive control ascorbic acid. The methanol extract has shown the low IC₅₀ value of 177.28 µg when compared to the Hexane (429.46 µg) and Acetone extract (247.46 µg) (Table 2). For the last decade, there is an increased search of natural antioxidants from the new sources due to the ill effects
Table 1. Qualitative Analysis of Whole Arial Part Extracts of Bryoniaepigae (Rottler).

<table>
<thead>
<tr>
<th>S/No</th>
<th>Tests</th>
<th>Hexane extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
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<tr>
<td></td>
<td>Alkaloids</td>
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</tr>
<tr>
<td>1</td>
<td>Mayer's</td>
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<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Dragon</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Wagner's</td>
<td>Negative</td>
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<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Hager's</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Phenolics</td>
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<tr>
<td></td>
<td>FeCl₂ Test</td>
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<td>Negative</td>
<td>Positive</td>
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<tr>
<td>3</td>
<td>Flavanoids</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>Lead acetate test</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
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<td>NaOH test</td>
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<td>Negative</td>
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<td>Ethyle acetate test</td>
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<td>Positive</td>
<td>Negative</td>
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<td>Salkowski's test</td>
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<td></td>
<td>FeCl₂ test</td>
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<td>Lead acetate test</td>
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<td></td>
<td>Pot. dichromate test</td>
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<th>Saponins</th>
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<td>12</td>
<td>Keller-kiliani test</td>
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</table>

![Graph showing antibacterial activity of whole arial part extracts of Bryonia epigaea (Rottler).](image-url)

**Figure 2.** Antibacterial Activity of Whole Aerial Part Extracts of *Bryonia epigaea* (Rottler).
Figure 3. Anti-bacterial efficiency of methanol extracts with different concentrations.

Figure 4. Anti-fungal efficiency of Methanol extracts with different concentrations.
Figure 5. Total phenol content of methanol extract.

Figure 6. % of DPPH activity for different extracts of Bryonia epigaea (Rottler).

Table 2. Antioxidant activity of Bryonia epigaea (Rottler).

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Name of the Extract</th>
<th>IC$_{50}$ Value (µg/ml)</th>
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<td>1.</td>
<td>Hexane Extract</td>
<td>429.46</td>
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<td>2.</td>
<td>Acetone Extract</td>
<td>247.76</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol Extract</td>
<td>177.28</td>
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of reactive oxygen species (ROS) production and oxidative stress. The oxidative stress has been linked to many diseases. The free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, inflammation, cardiovascular disorders, lung damage, reperfusion injury, atherosclerosis, neoplastic diseases and aging (Thomas and Kalyanaraman, 1997). The antioxidant activity of most of the plants is associated with their phenolic concentrations. There is a chance of a decrease in the antioxidant activity due to partial lacking of phenol constituents. These phenols and flavonoids have the functional groups with scavenging property (Kessler et al., 2003).

CONCLUSION

_Bryonia epigaea_ (Rottler) is the most instinctive plant having wide range of activities. Wide range of compounds were analyzed in methanolic extract. The antimicrobial efficiency of _Bryonia epigaea_ (Rottler) aerial part extracts showed good results against various pathogens Methanol extract showed good anti-oxidant activity. _Bryonia epigaea_ (Rottler) aerial parts were analyzed for its biological activities and further isolation and characterization of the compounds with biological activities will certainly add a valuable invention in the field of drug discovery.

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Conflicts of interest

Authors have none to declare.

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