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Munazza Maryam Niyazi
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science Akola, Maharashtra, India

ZH Khan
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science Akola, Maharashtra, India

Nazia D Khan
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science Akola, Maharashtra, India

SM Mular
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science Akola, Maharashtra, India

Correspondence
Munazza Maryam Niyazi
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science Akola, Maharashtra, India

Identification, purification and characterization of bioactive compounds present in *Bridelia ferruginea* and *Piper umbellatum*

Munazza Maryam Niyazi, ZH Khan, Nazia D Khan and SM Mular

Abstract
Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug discovery in the present study, principle phytoconstituents of *Bridelia ferruginea* and *Piper umbellatum* were identified in order to relate their presence with bioactivities of the plants, screening of the *Bridelia ferruginea* and *Piper umbellatum* were performed using standard method and resulted in the detection of presence of tannins, flavonoides, phenolics, saponins, steroids, cardiac glycosides and alkaloids. *Bridelia ferruginea* was found to contain all of seven bioactive compound where as the *Piper umbellatum* contained five compounds. It is evident from the study of *Bridelia ferruginea* the highest therapeutic efficacy processing majority of phytochemical classes of compounds. While *Piper umbellatum* recorded of the moderate therapeutic potential according to the high amount of active principles and their potential in medication, allelopathy, agrosystems daitery supplements or cosmetics industries.

Keywords: *Bridelia ferruginea*, *Piper umbellatum*, bioactive compound

1. Introduction
The tropical rainforest is home to a significant array of rich bio-resources and accounts for 25 % of plant-extractable accumulate different active principles, useful in treating various human or animal diseases. The long term use of herbs in medicine is a sure indication of their value and usefulness in the future. In modern medicine, the importance of medicinal plants is increasing with pharmaceutical and cosmetic industries increasingly using plant resources from rural or unpolluted areas. Nature has been a source of medicinal agents for thousands of years and generally produces many secondary metabolites which constitute important leads for the development of new environmentally friendly microbicides, pesticides, herbicides and many pharmaceutical drugs. Traditional societies in Africa and elsewhere have always used plants to promote healing and about 80 % of the world s population depends on the use of traditional medicine for health care (WHO, 1993). Therefore, such plants should be investigated to better understand their properties, safety and efficacy. As science advanced, however, it became possible to determine rigorously the active components of these extracts through painstaking and laborious chemical methods. This rational approach to the discovery of drugs inaugurated an era of bio-prospecting that is, raiding nature's storehouses of plant and microbiological life. Bio-Prospecting literally involves exploring the forests, diving in the oceans and digging in the dirt to obtain environmental samples. The study of the compounds discovered by these methods has become a major area of research in organic chemistry, biological science and has led to the isolation and identification of thousands of different structures, mostly extracted from plants and more recently from microorganisms, with the animal kingdom contributing rather sparsely to the total. Over the last few decades, the biological and pharmacological potentials of organic substances from many indigenous plants have been well understood. For instance, phenolic compounds have been associated with antimicrobial, anti-inflammatory, antiviral, and cytotoxic activities.
However, the bioactive constituents conferring these properties on many plant species have also been implicated in allelopathy. Allelopathy has been defined as the effect(s) of one plant on other plants through the release of chemical compounds in the environment. Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil

leachates and their derived compounds, can have allelopathic activity that varies over growing seasons (Rizvi *et al*., 1999) [4]. It has been indicated that phenolic acids are the most commonly occurring natural products noted for allelopathic activities have also included alkaloids, coumarin, flavonoids, saponins and volatile constituents of the essential oils as being allelopathic agents. Generally, the presence of different phytochemicals in crude plant extracts has been linked to the detrimental effects of leachates, root exudates or ecomposing residues of such plants on the other vegetation or succeeding crops (Mubashir and Wajaht, 2011) [3].

Phytochemical analyses of several species of medicinal plants and allelopathic activities of the crude chemical compounds on crops and plants have yielded positive results Of the different plant families studied, indicated that members of the Asteraceae family have great potential for inhibitory activities. Thus, the increased interest in the isolation and identification of the chemical compositions of organic products associated with biological activities with particular emphasis on germination, growth and yield of crops has stimulated research on plants having both medicinal and allelopathic properties.

2. Material and Methods

2.1 Medicinal plant

Bridelia ferruginea and *Piper umbellatum* were procured from commercial vendor in Akola district, Maharashtra and were authenticated by botany department of Shri Shivaji College, Akola. The plant material were dried under the shade then grinded and the herbal powder were ready to use.

2.2 Preparation of extracts

Fresh plant materials were collected for each plant and dried at room temperature in an aerated laboratory for three weeks. The dried materials were ground using a mill with 2 mm sieve attached to it to yield a fine powder. One hundred grams of each powder was weighed and macerated three times in 1000 mL of acetone for 48 hours. The mixture was filtered using Whatman filter paper No. 1 and the filtrate concentrated under reduced pressure by rotary evaporation (BUCHI Rotavapor R-200, Switzerland) at appropriate temperature. Residual solvent was removed by drying in air at room temperature (23-25 °C) and the extract weighed and stored at -20 °C until used. An aliquot of each crude extract obtained was used for phytochemical tests while the remaining fraction was kept for further studies.

2.3 Phytochemical screening

The concentrated residues from the acetone extracts were used to detect the secondary plant metabolites including alkaloids, flavanoids, steroids, saponins, glycosides, phenolics and tannins using standard methods with some modifications.

2.4 Test for saponins (Frothing test)

Saponins were tested by dissolving one half gram (0.5 g) of the crude extract in a test tube containing 3 mL of hot distilled water and then the mixture was shaken vigorously for one minute and persistent foaming observed indicated the presence of saponins.

2.5 Test for flavonoids (Cyanidine test)

One half gram (0.5 g) of the crude extract was dissolved in methanol and 2 mL of concentrated hydrochloric acid added. A spatula full of magnesium turnings was added and the mixture observed for effervescence. A brick red colouration observed indicated the presence flavonoids.

2.6 Test for steroids (Lieberman-Burchard test)

About one half gram (0.5 g) of the crude extract was dissolved in 0.5mL dichloromethane to give a dilute solution and then 0.5 mL of acetic anhydride added, followed by three drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

2.7 Test for tannins (Ferric chloride test)

One half gram (0.5 g) of the crude extract was dissolved and added to a tube containing 20 mL of boiling distilled water and then boiled for an hour. A few drops of ferric chloride was added and allowed to stand for proper colour development. A blue-black colouration indicated the presence of tannins.

2.8 Test for Alkaloids (Dragendorff's test)

The sample was dissolved in dichloromethane and then spotted on a thin layer chromatographic plate which was developed in 20 % hexane in ethylacetate. The presence of alkaloids in the developed chromatogram was detected by spraying with freshly prepared Dragendorff's reagent in a fume chamber. A positive reaction on the chromatogram indicated by an orange or darker coloured spot against a yellow background is confirmatory evidence that the plant extract contained alkaloids.

2.9 Test for cardiac glycosides

An extract of the plant was added to 2 mL of glacial acetic acid plus one drop of ferric chloride. The set up was underplayed with 1 ml of concentrated sulphuric acid. There was the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer which indicated the presence of cardiac glycosides.

3. Results and Discussion

After performing the analysis of bioactive compounds of the studied medicinal plants extracts, results obtained are as shown in Table. The phytochemicals, steroids, alkaloids, phenolics, Cardiac glycosides, tannins, saponins, were detected as present in the medicinal plants.

Table 1: *Bridelia ferruginea*

S. No.	Phyto-constitutes	Acetone extract
1	Saponins	+
2	Steroids	+
3	Tannins	+
4	Alkaloids	+
5	Cardiac glycoside	+
6	Flavonoides	+

Table 2: *Piper umbellatum*

Sr. No.	Phyto-constitutes	Acetone extract
1	Steroids	+
2	Alkaloids	+
3	Cardiac glycosides	+
4	Flavonoides	+
5	Tannins	+

4. References

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