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Comparative phytochemical and antimicrobial activities from leaf extracts of *Morus alba* and *Andrographis paniculata*

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Abstract: Due to widespread medicinal importance and wide region of *Morus alba and Andrographis paniculata*, the present work has been undertaken to study the phytochemicals and their antimicrobial efficacy in their leaf extracts. The leaves samples of both the selected plant were subjected to qualitative and quantitative phytochemical analysis for the presence of various secondary metabolites by various spectrophotometric techniques. Phytochemical screening revealed the presence of flavonoids, tannins, phlobatannins in *Andrographis paniculata* and tannins, reducing sugars, alkaloids, phlobatannins and saponins in Morus alba. The present investigation is also reveals that both the selected samples exhibit potent antimicrobial activity against *Escherichia coli*. So it is also proved as a source of certain antimicrobial molecules.

Keywords: *Morus alba*, *Andrographis paniculata*, Phytochemical, Antimicrobial

1. Introduction

From millions of years, plants have been a rich source of bioactive compounds and the use of plant derivatives has gained scientific and technological importance in these days. Medicinal plants are used to cure variety of diseases for long years. The aim of the study is to identify such plants with antimicrobial and antioxidant efficiency for controlling some diseases (Rang *et al.,* 2003). Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughout drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz *et al.,* 2004). These medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. According to world health organization (W.H.O) 80% of the

population of developing countries for their primary health care needs to depend on traditional medicines out of which mostly are plant based. Phytochemicals are natural bioactive compounds which are present in plants. These natural compounds work with nutrients and dietary fibers to protect animals and man against diseases. In this article a comparative study was conducted among *Morus alba* and *Andrographis paniculata* for their phytochemical and antimicrobial activities. Among the various plants used for medicinal purposes, the genus *Morus*, popularly known as mulberry, which belongs to the family *Moraceae* is well known for its medicinal properties. Three species are best known: *Morus alba, rubra, and nigra*. About 12-16 species of genus *Morus* are found in sub-tropical, warm and temperate regions of Asia, Africa and North America. *Mulberry* plant is one of the conventional herbs used in medicine (Wang *et al.*, 2013). *Mulberry* is commonly used as silkworm diet and an alternative medicine and reported to contain many flavonoid compounds and free radical scavenging effects. It contains many phenolic that can reduce cardiovascular disease. *Mulberry* showed high antioxidant activity in LDL oxidation assay. Mulberry leaves have been used for years to treat hyperglycemia, inflammation, cough, hypertension, cancer and fever (Ramesh *et al.*, 2014). *Andrographis paniculata* also called Kalmegh or King of bitter belong to family *Acanthecia*. It has been used to treat gastrointestinal track and upper respiratory infection, fever herpes, sore throat and a variety of other chronic and infectious diseases (Akbar, 2011). *Andrographis* is considered as the herb possessing an important "cold property" useful to treat the heat of body in fevers, and to dispel toxins from the body (Zhang and Tan, 2000). So the present study was done to evaluate the phytochemicals and antimicrobial activities of selected plants.

2. Materials and Methods

2.1 Sample collection

The leaf samples of *Morus alba* and *Andrographis paniculata* were collected from Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, in the state of Himachal Pradesh, India. The plant samples were properly washed and processed for authentication purpose.

2.2 Preparation of aqueous extract of *Morus alba* and *Andrographis paniculata*

The leaves of *Morus alba* and *Andrographis paniculata* were brought to the lab and were sundried so as to remove the moisture content. Once the samples were properly dried, they were

grounded to make a fine powder and used for making extract. For this 10 gm of each powder was dissolved in 100 ml of distilled water and kept in rotary shaker at 100 rpm for 72 h. The process was repeated until the complete extraction was done. After the complete extraction, the mixture was centrifuged at 4000 rpm for 10 min. Then supernatant was collected in china dish and allowed to evaporated to make a thin extract on a waterbath (95° C). After evaporation, the dried extracts were stored at 4° C in eppendorf tubes for further analysis.

2.3 Qualitative screening of phytochemicals

The prepared leaf extracts of *Morus alba* and *Andrographis paniculata* were tested for the presence of various secondary metabolites such as alkaloids, saponins, flavonoids, tannins, cardiac glycosides, reducing sugars and phlobatannins according to establish procedures (Trease and Evans, 1989; Sofowora, 1993; Parekh *et al.*, 2006 and Harborne, 1998; Aguinaldo *et al.*, 2007).

2.4 Quantitative screening of phytochemicals

After the proper qualitative screening the prepared plant extracts were screened for the quantitative phytochemical analysis. The total phenolic content of each plant extract were determined with the help of Folin- Ciocalteu reagent test. Total phenolic content of each plant extract were calculated from calibration curve of gallic acid (25-100 µg/ml) and expressed as mg gallic acid equivalents /gm weight of the extract. The total flavonoids content was measured by a colorimetric assay. Total flavonoid content of the extracts and fractions will be expressed as mg rutin equivalents (RU) per gram of sample (mg/g) (Mishra *et al.*, 2012)

2.5 Antioxidant analysis of plant extracts by DPPH method

Different volumes (2 – 20µl) of plant extracts were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1mM, 4 mg of DPPH in 100 ml of methanol) solution was added. The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\%RSA = Abs_{control} - Abs_{sample} / Abs_{control} \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + plant extract. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

2.6 Antimicrobial efficacy (Valgas *et al.*, 2007)

The surface of nutrient agar plates was swabbed with 100 µl of bacterial culture and spreading was done with L- shaped spreader properly. Then plates were allowed to dry under laminar air flow for 10-15 min. Then a hole with a diameter of 8 mm is punched aseptically with pre-autoclaved tip. Now 100 µl of each plant extracts was added into each well with different concentration. Then agar plates were incubated for 24 h at 37 °C. The antimicrobial agent diffuses into well and inhibited the growth of microorganism tested. DMSO and streptomycin was used as negative and positive control respectively.

3. Results and Discussion

3.1 Qualitative Phytochemical Analysis

The analysis and characterization of phytochemicals from plants is important to as certain their medicinal value. The present study reveals the presence of pharmacologically active phytocompounds viz. alkaloids, saponins, flavonoids, tannins, cardiac glycosides, reducing sugars and phlobatannins. Both the selected plant showed the presence of different types of phytochemicals in their aqueous leaf extracts (Table 1). The cardiac glycosides were found to be absent in both the plants.

Table 1:- Qualitative Phytochemical analysis of *Morus alba* and *Andrographis paniculata.* '+' indicate the presence and '-' indicates the absence

Phytochemicals	***Andrographis paniculata***	***Morus alba***
Alkaloids	-	+
Saponins	-	+
Flavonoids	+	-
Tannins	+	+
Cardiac Glycosides	-	-
Reducing Sugars	-	+

Phlobatannins	+	+

Phytochemicals found in leaf extracts of both the selected plants indicates its potential as an important source of medicine and also to improve the health of its users as a result of the presence of various compounds that are vital for good health. Several reports are available which indicates the presence of various phytocompounds in the *Morus alba* and *Andrographis paniculata.*

3.2 Quantitative Phytochemical Analysis

After the preliminary phytochemical screening, the qualitative phytochemical estimation was done for the quantification of total phenolic and total flavonoid content. The total phenolic content (TPC) was found to be 94.8 and 66.6 mg/GAE gm in *Andrographis paniculata* and *Morus alba* respectively whereas on the other hand the total flavonoid content (TFC) was found to be 82.2 and 54.2 mg/RU gm in both the selected plants i.e. *Andrographis paniculata* and *Morus alba* respectively. Both TFC and TPC were found to be highest in the case of *Andrographis paniculata.* Nagaraja and Chandrashekhar, 2014 reported the presence of various phytochemicals present in the *Andrographis paniculata*.

Table 2:- TFC and TPC content of *Andrographis paniculata* and *Morus alba*

Phytochemical	***Andrographis paniculata***	***Morus alba***
TFC (mg/RU gm)	82.2	54.2
TPC (mg/GAE gm)	94.8	66.6

3.3 Antioxidant Analysis

The radical scavenging capacity of the selected plant leaf extracts with different concentrations was tested using the ‘stable’ free radical, DPPH. Table 1 shows the ability of both the selected plants to scavenge DPPH radical. It was observed that both the aqueous extracts exhibited a significantly higher radical scavenging activity. The highest radical scavenging effect was found

to be highest in *Morus alba* as compared to *Andrographis paniculata.* The IC_{50} value was found to be 67.14 µg/ml and 53.41 µg/ml in *Morus alba* and *Andrographis paniculata* respectively.

3.4 Antimicrobial activities of selected plants

The antibacterial activities of aqueous leaf extract of *Morus alba* and *Andrographis paniculata* were determined against *E.coli*. The 100 µl of each plant extract was used for each bacterial strain. Moreover, streptomycin was used as positive control for overall experiment. The aqueous solution of DMSO (dimethyl sulfoxide) was used as negative control. All the sampled populations found to be shown the inhibitory effect. The antibacterial activities were measured as zone of inhibition in mm. All the prepared extracts show good zone of inhibition against all the selected pathogen. Narayanan *et al.,* 2015 revealed that the leaf extract of *Andrographis paniculata* has shown the maximum antibacterial activity against *Escherichia coli* with zone of inhibition of 19.4mm. The antibacterial activity of all the medicinal plant extracts depends on the presence of different concentrations of secondary metabolites (Soetan *et al.,* 2005). Our results support the previously published reports that the gram positive bacteria are more sensitive to plant extracts when compared to that of the gram negative bacteria. Both the plants extracts prepared from the polar solvents possess excellent antimicrobial property that can be used as a potential source of antibiotics.

Table 3:- Antimicrobial activities of *Morus alba* and *Andrographis paniculata*

Bacterial strain	***Morus alba***	***Andrographis paniculata***	**Antibiotic (mm)**
E. coli	22	20	24

. 4. Conclusion

Medicinal plants are known to play significant role in drug development as plant produce a wide range of secondary metabolites. Plant derived medicines are widely used because they are safer than the synthetic alternatives. Medicinal plants such as *Morus alba* and *Andrographis paniculata* has medicinal values. In the present study, leaf samples of *Morus alba* and *Andrographis paniculata* were screened for phytochemical and antimicrobial analysis. The result of the present study suggests that *Morus alba* and *Andrographis paniculata* have moderate potent antioxidant activity of free radical scavenging activity. The great antioxidant potential will be will be of immense benefit from the consumption of this plant/extract. The plant extract was

effective against *E.coli* and zone of inhibition in *Morus alba* and *Andrographis paniculata* was found to be 22 and 20 mm respectively. The finding of this study support the view that some medicinal plant are promising source of potential antimicrobial, phytochemical and antioxidant that may be efficient as preventing agent in some diseases .The plant plays very important role in the field of medicine and pharmaceutical and also treat infectious diseases. Both the plants i.e. *Andrographis paniculata* and *Morus alba* taken under study can be used as potent drugs exploiting the anticancer, anti-infection, antidiabetic activities of the plant. This study is to aware peoples towards the application of drugs at affordable cost. Further research is needed to isolate, identify, and characterize the structure of bioactive constituents.

5. References

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