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Screening of Phytochemicals, antioxidant and silver nanoparticles biosynthesizing capacities of some medicinal plants of Nepal

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Abstract: In this study, three plants i.e. *J. adhatoda, A. vulgaris* and *P. guajava* native to Pashupati area and Jorpati of Nepal were screened to evaluate the phytochemical and antioxidant activities due to their high medicinal value. The dried leaves were extracted with three solvents: hexane, methanol and distill water. The extracts were subjected to various phytochemical tests. The test confirmed the presence of various phytochemicals. The antioxidant activity of the methanol extracts were evaluated by FRAP assay. The antioxidant activity of *J. adhatoda* was found to be 0.794mM Fe (II)/L, *A. vulgaris* to be 0.949 mM Fe (II)/L and *P. guajava* was found to be 2.035 mM Fe (II)/L. Fresh aqueous leaf extracts of *A. vulgaris* and *J. adhatoda* were used for synthesis of silver nanoparticles. The biosynthesized AgNPs were characterized by Transmission Electron Microscope (TEM).

Keywords: Phytochemicals, *Justicia Adhatoda, Artemisia Vulgaris, Psidium Guajava,* Antioxidant Activity, FRAP, Silver Nanoparticles

1. Introduction

Plants have a great importance in our lives because they fulfill our basic needs for food, shelter, clothing, fuel, ornamentals, flavoring and medicine. The medicinal value of these plants is mainly due to the presence of some chemical active substances called phytochemicals. The phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health. It has been claimed that phytochemicals have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effect to mention but a few. The phytochemical constituent of a plant will often determine the physiological action on the human body [1].Antioxidants protect cells against damage caused by molecules known as free radicals. The antioxidant effects in plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [2]. The effectiveness of phytochemicals in the treatment of various diseases may lie in their antioxidant effects [3]. Thus, the present study was carried out to evaluate the phytochemicals and antioxidant activity of following three plants with medicinal values.

Justicia adhatoda, Acanthaceae family and locally called "Asuro" is a perennial, evergreen and highly branched shrub (1-2.5m high) with unpleasant smell and bitter taste. It has opposite ascending branches with white, pink or purple flowers [4]. The plant grows wild in abundance all over Nepal, Sri Lanka, India, and the Pothohar region of Pakistan, particularly in the pharwala area. It is widely used in the treatment of cough, bronchitis, asthma and common cold [5].

Artemisia vulgaris, family Asteraceae or Compositae, commonly called "Titepati" is widespread in temperate areas (South Europe, North Africa, North America and Asia) [6]. It is slightly toxic as its prolonged dosage can damage the nervous system [7]. All parts of the plant are anti-inflammatory, antispasmodic and used in the treatment of women's complaints [8].

Psidium guajava, family Myrtaceae, is commonly known

as poor man's apple and called "Amba" in Nepali. The tree is common throughout the world specially all warms areas of tropical America, West Indies, Asia, Africa and other subtropical countries including Nepal. Commercially the fruit is consumed fresh or used in the making of Jams, Jellies and Paste. *P. guajava* a widely distributed evergreen plant which has several medicinal uses in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhea, abdominal distention and gastric pain [9]. Ground leaves are used as poultice, ripe fruits are good laxative and the stem is good astringent, is recommended for gout. The root bark is successfully employed in diarrhea of children in the form of concentrated decoction [10-12].

Though there are lots of medicinal plants in Nepal, studies on those plants for their medicinal uses were not conducted very often. So far a few species of Nepalese plants have been studied for their antioxidant activity [13, 14]. To our best knowledge, there have been no previous studies on silver nanoparticles biosynthesizing capacities of *A. vulgaris* and *J. adhatoda* in Nepal. Therefore, this study was also conducted to evaluate the silver nanoparticles biosynthesizing capacities in these two plants.

2. Materials and Methods

2.1. Collection of Plant Materials

J. adhatoda and *A. vulgaris* leaves were collected from Pashupati area and *P. guajava* leaves were collected from Jorpati area in Kathmandu, Nepal. All plants were authenticated by National Herbarium and Plant Laboratories, Godawari, Nepal.

2.2. Preparation of Plant Extract

The shade dried leaves of *J. adhatoda; A. vulgaris* and *P. guajava* were blended and made into fine powder. Each leaf powder was extracted with three solvents; Hexane, Methanol and Water. The extraction was run in soxhlet apparatus and extracts were collected in a beaker. The solvent was allowed to evaporate in Rotatory evaporator till complete evaporation was achieved. This gave the final leaf extract which was used for further experimental procedure.

2.3. Phytochemical Screening

The phytochemical screening was carried out according to the procedure given by Prof. I. Ciulei [15].

2.4. Antioxidant Activity

The antioxidant activity was evaluated by FRAP assay [16]. The FRAP reagent was prepared by mixing acetate buffer of pH 3.6 (300 mM), TPTZ (tripyridyltriazine) solution of 10 mM and ferric chloride solution of 20mM in the ratio of 10:1:1. Antioxidant activity was calculated with the standard calibration of ferrous sulfate. The leaf extracts (5 mg/ml) was prepared by adding methanol and was used as sample. Finally, absorbance was taken at 593 nm keeping the temperature 37 °C.

2.5. Statistical Analysis

All data were computed from the mean of three independent experiments and expressed as mean ± SD. Statistical analysis was carried out using GraphPad Prism 6 software.

2.6. Synthesis of Silver Nanoparticles

10^{-2} M $AgNO_3$ was prepared and stored in color bottle. 5 ml of leaf extract was taken in a conical flask to which 5 ml of 10^{-2} M $AgNO_3$ was added along with 2.5 ml of 25% ammonia and stirred. The conical flask was incubated at room temperature for 24 hours and observed the color change. The appearance of dark yellowish-brown color indicated the synthesis of silver nanoparticles.

2.7. Characterization of Silver Nanoparticles

Transmission Electron Microscope (TEM) was used to characterize the Ag nanoparticles.

3. Results and Discussion

The paper describes the phytochemical, antioxidant and silver nanoparticles biosynthesizing capacities of three indigenous medicinal plants of Nepal**.** The phytochemical screening of the plants revealed some differences in the constituents of the three plants tested *J. adhatoda* tested positive for all the phytochemicals tested except carotenoids and emodins. *A. vulgaris* showed the absence of anthracenosides, anthocyanosides, carotenoids and Emodin while *P. guajava* tested negative for terpene carotenoids, coumarins reducing compounds and quinones. *J. adhatoda* was found to contain highest number of phytochemicals and *P. guajava* contains the least number of phytochemicals among three plants. The results of phytochemical screening are shown in tables 1-3.

Table 1. *Phytochemical screening of hexane extracts*

SN	Groups of compounds	*A. vulgaris*	*J. adhatoda*	*P. guajava*
1.	Volatile oils	+	+	-
2.	Basic alkaloid	+	+	+
3.	Sterols and triterpenes	+	+	-
4.	Carotenoids	-	-	-
5.	Fatty acids	+	+	+
6.	Coumarins	+	+	-
7.	Flavones aglycones	+	+	+
8.	Emodins	-	-	-
9.	Quinones	+	+	-

Table 2. *Phytochemical screening of methanol extracts*

SN	Groups of compounds	*A. vulgaris*	*J. adhatoda*	*P. guajava*
1.	Polyphenols	+	+	+
2.	Reducing compounds	+	+	-
3.	Alkaloid salts	+	+	+
4.	Glycosides	+	+	+
5.	Quinones	+	+	-
6.	Anthocyanosides	-	+	+
7.	Anthracenosides	-	+	+
8.	Coumarin derivatives	+	+	+
9.	Flavonic glycosides	+	+	+

Table 3. *Phytochemical screening of aqueous extracts*

SN	Groups of compounds	*A. vulgaris*	*J. adhatoda*	*P. guajava*
1.	Polyoses	+	+	+
2.	Saponins	+	+	+
3.	Alkaloids	+	+	+

The highest antioxidant activity was found in *P. guajava*. The other two plants showed almost similar activities. The antioxidant activity of *P. guajava* was 2.035 mM Fe(II)/L, *A. vulgaris* and *J. adhatoda* were found to be 0.949 mM Fe(II)/L and 0.794 mM Fe(II)/L respectively (table 4).The phenolic compounds containing free hydrogen are responsible for these antioxidant activities [18]. It become evident that the antioxidant activities of all the extracts are due to the presence of flavonoids and polyphenols in all the plants. Many reports have been published in the literature on the phytochemical screening and antioxidant activities of these plants[19-21]. However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants. The present study also correlates with the results of previously reported with little variations.

Table 4. *Antioxidant activity of plant extracts in 5 mg/ml concentration*

Plant extract	Concentration (mg/ml)	Antioxidant activity [mM Fe(II)/L]
J. adhatoda	5	0.794 ± 0.005
A. vulgaris	5	0.949 ± 0.021
P. guajava	5	2.035 ± 0.085

The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media [22]. Among many techniques of synthesizing silver nanoparticles, plant-mediated biological synthesis of nanoparticles is important due to its simple experimental procedure and eco-friendliness[23-25]. Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens, compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Many reports have been published in the literature on the biogenesis of silver nanoparticles using several plants extract, particular Neem leaf broth *Aradirachta* [26], leaf broth natural rubber[27], *Aloe vera plant extracts* [28], starch [29] etc. The biogenesis of silver nanoparticles using plant extracts from Nepal has not been investigated so far. Here biosynthesis of silver nanoparticles by the aqueous extract of *J. adhatoda, and A. vulgaris* were investigated. Biosynthesis of silver nanoparticles by each plant extracts is confirmed by color change from clear to pale yellow brown after 24 h of reaction period. It is due to the surface plasmon resonance phenomenon [17] (fig.1).

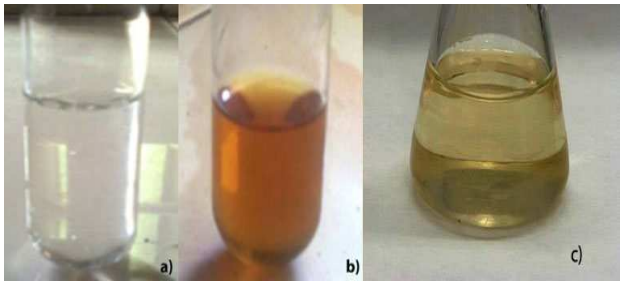

Figure1. *a) Solution of silver nitrate (10-2 M) before addition of plant extract, (b) after addition of plant extract (A. vulgaris) and (c) After addition of plant extract (J. adhatoda). There was the change in color after addition of plant extracts in silver*

Silver nanoparticles synthesized with *A. vulgaris* and *J. adhatoda* leaf extracts were subjected to TEM. *A. vulgaris* revealed the formation of nanoparticles in 200 nm. The size distribution was wide and the particles with smallest diameters were formed with no significant aggregates. *J. adhatoda* showed the formation of nanoparticles in 500 nm. Although particles were fine, the size distribution was narrow and smaller aggregates were formed (fig 2).

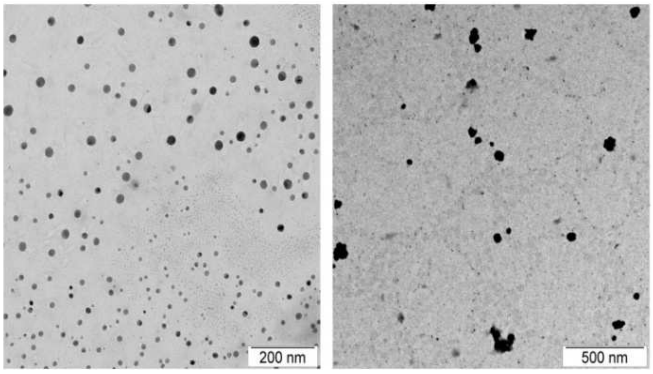

Figure2. *Transmission Electron Micrograph of AgNPs by A. vulgaris (left) and J. adhatoda (right)*

4. Conclusion

Phytochemical screening and antioxidant activity of three medicinal plants have been successfully carried out. A wide range of phytochemicals is present in all plants. *P. guajava* showed highest antioxidant activity followed by *A. vulgaris* and *J. adhatoda* showing almost similar activities. Also, silver nanoparticles from *A. vulgaris* and *J. adhatoda* has been synthesized and characterized by TEM. Among these two plants, *A. vulgaris* revealed the better result.

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Recommendations

Antimicrobial activities of these plants can be studied. Characterization of silver nanoparticles by EDX and antimicrobial assay of silver nanoparticals can be done.

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