Qualitative and Quantitative Phytochemical Screening of Azadirachta indica Juss. Plant Parts

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ABSTRACT

Azadirachta indica which is commonly known as neem plant has one of the most promising medicinal properties having a wide spectrum of biological activity. Fresh matured leaves, stem-bark and seeds of A. indica (neem) were collected, air dried and aqueous-extract was used to screen for some active chemical constituents. Phytochemicals of neem plant parts were extracted and screened both qualitatively and quantitatively. Among the qualitative tests done for the presence of secondary metabolites; alkaloids, saponins, terpenoids, flavonoids were found to present in all of the tested parts whereas steroids, polyphenols and tannins were present only in leaves and stem-bark. Glycosides and coumarins are absent in all of the tested parts. Quantitative screening was also done by using gravimetric method. Alkaloids were found in all the parts of A. indica with the highest amount of alkaloids were obtained in stem-bark (12.8%) and lowest in leaves (10.67%). Highest flavonoids percentage was revealed in leaves (13.8%) and lowest flavonoids in stem-bark (12.8%). Similarly, seeds (2.53%) contained saponins % while it was lowest in stem-bark (2.50 %). Terpenoids % were similar in both i.e. leaves and stem-bark (13.13%), whereas lowest in seeds (12.77%). More research on secondary metabolites will be helpful to the chemical industries to produce plant-based chemicals and minimize the environment degradation from different synthetic chemicals.

KEYWORDS :-

Azadiracta indica, Phytochemicals, Metabolites

INTRODUCTION :-

Azadirachta indica Juss. is one of the traditional medicinal plants in the south asia and each part of the tree has some medicinal properties. A.indica commonly known as Tree of wonder (Khetarpal, 2010) or Neem is a wonder tree in the mahogany family Meliaceae. Neem also has been called “nature’s drug store” (Paul, Prasad & Shah, 2011). Neem is one of the fast-growing trees which can reach upto a height of 14- 18 metres, and sporadically 35-40 m. It is deciduous tree with wide branching and shedding of its leaves during winter. Neem is drought resistance tree which can thrive well in trophical and sub-trophical climates. The major active constituents extracted from neem seed are Azadirachtin, Salanin and Nimbin. Nimbin was the first chemical limonoid isolated from neem tree. Extract of seeds, leaves and bark of the Neem tree contain Azadirachtin which has been reported to have strong biological activities against insect pests, but with very low toxicity to mammals and the environment (Makeri et al., 2007; Umar et al., 2002). Subsequently, more than 150 bioactive chemical compounds have been isolated from various neem tissues (Brahmachari, 2004). Neem contains active substances in almost every parts i.e. seeds, leaves, roots, bark, trunk and branches and have multiple medicinal properties (Khan & Aslam, 2008). A. indica leaves showed presence of saponins, flavonoids, phenols, tannins,

alkaloids, glycosides, proteins, triterpenoids, carbohydrates and alkaloids (Pandey et al., 2014). Medicinal plants are derived from plant belonging to different families and either utilized as plant extracts, essentials oil or both (Srinivasan et al., 2001). Plant extracts like bark, stem, root, flowers, fruits, rhizomes and stem plants consist of several bioactive compounds that are antimicrobial and anti-fungal (Rahmatullah, 2009). Medicinal plants have more than one chemical as an active principle responsible for their biological properties (Khanal, 2018). Phytochemicals are chemical compounds produced by plants (Brielmann, 2006), generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals (Chukwuebuk & Chineya, 2015). Phytochemicals are chemical substances generated by the plants which helps to counter them against fungi, bacteria, virus inflammation and protects from exhaustion of insects and other animals. The name is derived from Greek (phyton) 'plant' which is thought to be responsible for protective health benefits (Webb, 2013). Phytochemicals are chemicals of plant origin (Breslin, 2017). Naturally phytochemicals occur in medicinal plants, leaves, grains, vegetables and roots etc. They are primary and secondary compounds. Primary compound includes chlorophyll, proteins and sugars whereas in secondary compounds flavonoids, alkaloids, sterols, terpenoids, flavonoids, saponins, tannins, volatile oils, etc. (Motalab, 2011). Some phytochemicals have been used as toxicants and also as a traditional medicine. De Albuquerque (2006) states that approximately 20% of known plants have been used in pharmaceutical studies, impacting the factor they can be used in various research fields of agriculture and can be used as alternative medicines to protect plants from fungi, insects, bacteria and virus. The increasing environmental degradation due to burgeoning chemical products industry has cause increase in alarm bells all over the world. So, to minimize the issues of chemical products medicinal Plants' chemicals can be one of the best possibilities in today's condition.

Materials and Methods:-

Collection of Plants

The neem parts i.e. leaves, seeds, stem-bark were collected from Gaidakot Municipality, Nepal and dried for screening of secondary plant metabolites. They were collected either randomly or by following leads supplied by local healers in the study area on Jan 2, 2021. The research was carried out at Ecology laboratory condition of Paklihawa Campus during 3rd to 20th February in 2021 at 27.73o north and 84.39o east. Leaves, seeds and stem-bark were used for the experiment because leaves are active in photosynthesis and production of metabolites is high as compared to other parts (Ghorbani, 2005).

Extraction, Cleaning and Drying of Plant Materials

Prior extraction, leaves, stem-bark and seeds were cleaned 2 to 3 times with running water and once with sterilized distilled water then surface sterilized with 1% mercuric chloride. The materials were dried under shade at room temperature (30±50 c) for 10 days.

Preparation of Crude Powder

After about 10 days of shade drying, well dried plants parts were powdered by using electric mixture .Then product was subjected to mass sieving to obtain fine powder. Powder was kept in a plastic jar with air tight lid and store for required period.

Preparation of Stock Solution

100 gm crude powder of each collected parts were soaked in 1000 ml of distilled water separately and left for overnight in air tight plastic bottle for maceration. Mixture was filtered in Whatman filter paper No. 42 boiled for 5 min in heating mantle and allowed for cooling by keeping in desiccators. Stock solution was kept in the refrigerator at 4 oC for future use.

Qualitative Phytochemicals Analysis

Preliminary qualitative phytochemicals screening was carried out following standard protocols (Shrestha et al., 2015).

Test for Alkaloids

Mayer's reagent was used to test alkaloids. 2 ml of botanicals extract was taken in a test tube and 2-3 drops of Mayer's reagent added on it. Presence of alkaloid was indicated by the appearance of green color precipitate in the solution. Wagner's test was done by using Wagner's reagent. When few drops of Wagner's reagent added in test tube containing 2 ml of extract, the appearance of brick color precipitate indicated the presence of alkaloids.

Test for Flavonoids

Alkaline reagent test: 2 ml of botanicals was taken in a test tube and 2 ml of sodium hydroxide (2% w/v) solution was also added on it. An intense yellow color appeared in the test tube. On addition of few drop of dilute hydrochloric acid, it was colorless which indicated the presence of flavonoids. For Shinoda Test, 2 ml of botanical extract was taken in a test tube. 5 drops of Hydrochloric acid and 0.5gm of magnesium pieces was added on it. Pink color was observed in the solution containing flavonoids.

Test for Saponins

Foam test: The extract solution was diluted with distilled water and taken in a test tube. There was a suspension formed for minutes. Two cm layer of foam indicated thepresence of saponins.

Test for Terpenoids

Crude extract was dissolved in 2ml of chloroform and was evaporated to dryness. To this, 2ml of concentrated H2SO4 was added; a reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for Glycosides

Salkowski's test: Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown color indicates the presence of steroidal ring, i.e., glycone portion of the glycoside. Keller-Kilani test: Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was poured into another test tube containing 2 ml of Concentrated H2SO4. A brown ring at the interface indicates the presence of cardiac glycosides.

Test for Polyphenols and Tannins

Crude extract was mixed with 2 ml of 2% solution of FeCl3. A blue green or blue-black coloration indicated the presence of polyphenols and tannins.

Test for Steroids

Crude extract was mixed with 2 ml of chloroform and concentrated H2SO4 was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H2SO4 and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Test for Coumarins

Extract solution is concentrated to yield a residue. Dissolve residue in hot water. After cooling divide solution in two test tubes. To one test tube add 10% (w/v) Ammonium Hydroxide. Other test tube was used as control. Fluorescence color was indicating the presence of coumarins.

Quantitative Test of Phytochemicals

Quantitative test of phytochemical was done by the gravimetric method described by Harborne, (1973):

Test for Flavonoids

The powdered sample i.e. 5 gram was placed into a conical flask with 100 ml of allowed to cool before filtered into Whattman No. 42 filter paper. Aqueous layer was discarded and filtered with pre-weighted filter paper. Residue of filter paper was dried in an oven for 30 minutes at 600C. Weight of flavonoids was

calculated by using following formula.

% Flavonoid = W2 − W1 / Weight of Sample × 100

Where,

W1 = weight of empty filter paper

W2 = weight of paper + Flavonoid extract

Test for Alkaloids

5 gram of sample dust was dissolved in 100 ml of 10% acetic acid. It was Well shook and left for 4 hours. The solution was then filtered in Whatman No. 42 filter paper. Filtrate was evaporated to 1⁄4th of its original volume using hot plate with magnetic stirrer. Concentrated Ammonium hydroxide (NH40H) was added drop wise to precipitate the alkaloid content. Solution was filtered again and washed with 1% NH4OH. Filter paper containing precipitate was dried in oven at 600 c for 30 minutes and weighed after allowed to cool for few minutes.

Alkaloid % = W2− W1 / W1 × 100

Where,

W1= weight of empty filter paper

W2=Weight of paper+ alkaloid precipitate

Test for Terpenoids

Dried plant extract 10 gram (Wi) was taken and soaked in 90 ml of ethanol (Indumathi et al., 2014).The extract after filtration was mixed with 10 ml of petroleum ether and again filtrated using separating funnel. The extract was waited for its complete drying and measurement is taken (Wf). The yield (%) of total terpenoids contents was measured by the formula:

Total terpenoids = Wi – Wf / Wi × 100

Where,

Wi= dried plant extracts,

Wf= extracts after drying

Test for Saponins

The plant extract i.e. 25 ml was placed in a round bottom flask.100 ml of 50% alcohol was added and boiled for 30 minutes and filtered while hot through a filter paper. 2 gram of charcoal was added to the filtrate and it is boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate the saponins.

% of true saponins = W2−W1 / W1 × 100

Where,

W1=Weight of filter paper

W2=Weight of residue

Research Design and Data Analysis

The recorded data entry was done in MS Excel, followed by tabulation and data arrangement. Statistical analysis was done by using data analysis tools like R Stat 4.0.4, GEN Stat (18th edition) etc. The data were subjected to Analysis of Variance (ANOVA), mean separation by DMRT (Duncan Multiple Range Test) at 5% level of significance. The experiment was conducted in Completely Randomized Block Design.

Result and Discussion

Preliminary Qualitative Phytochemical Analysis

The phytochemical study revealed the presence of various phytochemicals in the methanolic extracts of different medicinal plants. None of the tested neem parts had all the phytochemicals i.e. alkaloids, saponins, flavonoids, terpenoids, glycosides, polyphenols, tannins, steroids and coumarins. The presence of Flavonoids was indicated by alkaline reagent test and shinod's test in all of tested parts. Presence of foam indicates that there was presence of flavonoids in all tested parts of neem. Terpenoids was seen present in all of them and indicated by reddish brown coloration. Polyphenols and tannins were present in leaves and stem-bark whereas absent in seeds and indicated by blue green color. Similarly, steroids were indicated by greenish coloration and absent only in seeds (Table 1). Unlike the other phytochemicals glycosides and coumarins are not found in A.indica plant parts. Phytochemical study revealed that leaves contained alkaloids, flavonoids, saponins, terpenoids, polyphenols and tannins which corresponded with the result of Uwague (2019) and Akange et al. (2019). Neem bark has shown the presence of alkaloids, saponins, flavonoids, terpenoids, polyphenols, tannins and steroids in extract where the result was in contrast with Sharma, Dua & Srivastva (2014). Neem seeds have reflected the presence of alkaloids, saponins, flavonoids and terpenoids, only, but as comparative to earlier studies there was only presence of alkaloids (Bigoniya, Singh & Srivastava, 2012). The presence of phytochemicals such as alkaloids, flavonoids, saponins, glycosides, steroids, terpenoids, polyphenols and tannins reveals that plant parts exhibit medicinal as well as pharmacological activities.

Quantitative Test of Phytochemicals of Neem Plant Parts

Quantitative analysis of phytochemicals was done by the gravimetric method as described by Harbone (1973). Quantitative screening of alkaloids, flavonoids, terpenoids and saponins was done in the selected plant parts of A. indica and data is shown in the Table 2. The highest alkaloids percentage was observed in stem-bark (10.77%) followed by seeds (10.73%) and leaves (10.67%) which are significantly on par with each other. Neem leaves gave considerably maximum flavonoids percentage (13.8%) than all other parts, followed by seeds (13.1%) and stem-bark (12.8%) which is significantly on par with each other. Similarly, seeds contain higher amount of saponins (2.53%) band lowest was observed in leaves (2.50%) as compared to bark (2.43%). Similarly, higher terpenoids percentage was revealed in leaves (13.13%) and bark (13.13%) of A. indica and lowest was seen in seeds (12.77%) (Table 2). According to the Ani and Okolie (2018), the highest concentration of alkaloids %, flavonoids %, terpenoids %, saponins % was seen in the leaves of A. indica than the Stem-bark and root. Biu et al. (2009) reported that aqueous leaf extract of A. indica possesses higher amount of saponins and low quantity of alkaloid which corroboration with the result.

Table 1: Qualitative phytochemical screening of A. indica plant parts

Tests	Leaves	Seeds	Stem-Bark
lkaloids test	+	+	+
Flavonoids test	+	+	+
Saponins test	+	+	+
Terpenoids test	+	+	+
Glycosides test	–	–	–
Polyphenols and tannins test	+	–	+
Steroids test	+	–	+
Coumarins test	–	–	–

Note: (+) = presence, (–) = absence

Table 2: Quantitative analysis of phytochemicals present in different medicinal parts of Azadirecta Indica

Plant Parts	Alkaloids	Flavonoids	Saponins	Terpenoids
Leaves	10.67±0.46	13.8±0.17	2.43±0.32	13.13±0.5
Seed	10.73±0.29	13.1±0.08	2.53±0.14	12.77±0.11
Stem-Bark	10.77±0.11	12.8±0.15	2.50±0.28	13.13±0.41
Grand mean	10.2	13.2	2.49	13.01
SEM	0.95	2.84	0.92	1.73
LSD	2.32	6.94	2.26	4.25
CV%	10.9	26.3	45.5	16.4

Means followed by the same letter in a column are not significantly different by DMRT at 5% confidence level.

LSD = Least significant difference. SEM = Standard error of mean. CV= Coefficient of variation

Conclusion

Plants are a source of large amount of secondary metabolites which are claimed to possess the bioactive compounds which are responsible for their antibacterial, antifungal, antifeedant, repellent, and pesticidal properties. Medicinal plants are one of the best alternatives to minimize the use of chemical-based fungicides. The results revealed the presence of medicinally important chemicals in the A. indica plant parts studied. Among the various qualitative tests done for the presence of secondary metabolites (alkaloids, flavonoids, saponins, terpenoids, glycosides, polyphenols, tannins, coumarins and steroids), alkaloids, flavonoids, saponins and terpenoids were found to present in all the tested parts whereas polyphenols, tannins and steroids were only present in leaves and stem-bark. Glycosides and coumarins were absent in all of the tested metabolites. Quantitative screening of alkaloids, flavonoids, saponins and terpenoids of A. indica leaves, stem-bark and seeds was done. Different phytochemicals percentage also differed significantly in different parts of A. indica. Higher alkaloids, flavonoids, saponins and terpenoids percentage was revealed in stem-bark, leaves, seeds and both leaves &stem-bark respectively. Many evidences gathered in previous studies have confirmed the presented phytochemicals are bioactive. The extracts from the plants can be good source of drugs for pharmaceutical industries. Thus, it also claims that the traditionally medicinal can be recommended and

suggest for further research to identify the active constituents of the Azadiracta indica. So, this can be one of the alternatives to utilize the phytochemicals of the medicinal plants and replace the hazardous chemicals in the environment.

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