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Phytochemical analysis and antioxidant activity of *Ipomoea aquatica* from Ghodaghodi wet land area, Nepal

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Abstract
Ipomoea aquatica is a green leafy vegetable; rich source of secondary metabolites, vitamins and amino acids with many health benefits. The present study was accomplished to analyze phytochemical constituents and antioxidant activity of hexane and methanol extracts of *Ipomoea aquatica* from Ghodaghodi wet land area, Nepal. For this purpose, first the powdered plant was extracted successively with hexane and then methanol. Thus obtained concentrated extracts were subjected to preliminary phytochemical screening following standard protocols. The antioxidant activities of extracts were evaluated by 2, 2-diphenyl -1-picrylhydrazyl (DPPH) radical scavenging assay. Ascorbic acid was used as standard antioxidant. Phytochemical screening of methanol extract of *Ipomoea aquatica* showed presence of wide array of natural bioactive chemical constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolics, saponins, quinones, tannins, terpenoids, proteins and amino acids etc. However, relatively less amount of phytochemicals like flavonoids, phenolic compounds, terpenoids, tannins were found to be present in the hexane extract implying that methanol is better solvent for phytochemical extraction. Similarly, in the hexane extract alkaloids, carbohydrates, glycosides, coumarins, saponins, quinones, protein and amino acids were absent. However coumarins were absent in both extracts of *Ipomoea aquatica*. The hexane extract showed less antioxidant activity (IC_{50} = 70.47µg/mL) whereas for methanol extract IC_{50} = 49.49 µg/mL. For standard antioxidant ascorbic acid IC_{50} = 53.99 µg/mL. The present study demonstrated higher antioxidant activity of methanol extract of *Ipomoea aquatica* was due to wide range of phytochemicals extracted in methanol in comparison to hexane as a solvent. This research also justifies *Ipomoea aquatica* as antioxidant rich green leafy vegetable available in wild nature.

Keywords: Antioxidant activity, 2, 2-diphenyl -1-picrylhydrazyl (DPPH) assay, hexane extract, *Ipomoea aquatica*, methanol extract, phytochemical screening

Introduction
In Nepal, traditional use of plant resources for medicinal purpose has a long history and is gaining popularity due to lack of side effects, easy availability and in many circumstances it is the only source of health care to poor communities and at least 1,600 to 1,900 plants species are being used traditionally for medicinal practice in Nepal [1].

I. aquatica is a green leafy, flowering vine. It is a tender, trailing or floating over the water surface or the marshy land. Its stem is 2-3 m or more long, rooting at the nodes, and they are hollow and can float over the water surface. The leaves vary from typically sagittate to lanceolate, 5–15 cm long and 2–8 cm broad. The flowers are trumpet-shaped, white in colour. *I. aquatica* belongs to Convulvulaceae family and known as water spinach, morning water glory or kalami saag. The plant is also widespread in Asia, Africa, Australia and America and is supposed to be originated in China [2]. Traditionally, for a long time *I. aquatica* has been used as animal feed and is common food eaten by all social groups of people as green leafy vegetable. Further the plant is used against various disorders such biliousness, bronchitis, diabetes, gastrointestinal disorder, liver malfunction, constipation, as well as in the treatment of the arsenic and the heavy metal poisoning. Medicinal properties of this plant have been used to cure nosebleed, high blood pressure, skin disease, to lessen inflammation, used in nervous and general debility of female in Assam [2-3].

I. aquatica contain a plethora of useful phytochemicals for humankind like; vitamins, minerals, proteins, carbohydrates, glycosides, fibers, carotenes, flavonoids, phenolic and polyphenolic compounds [4]. Extensive literature surveys of *I. aquatica* showed this plant has hypoglycaemic activity along with good antioxidant and antimicrobial activity. It was also found that most of the people all over the world include water spinach in food supplements [2-5]. In Nepal only some ethnobotanical survey had been carried out regarding to this plant

emphasizing on its distribution, habitat and local uses but tremendous amount of research work had been publish arena outside Nepal. Koirala *et al.* in 2019 have done research in 8 different types of wild vegetables along with *I. aquatica* to evaluate antioxidant activity, total phenolic and flavonoids content [6]. Many researches had conducted on phytochemicals screening and biological activities of whole plant extracts of *I. aquatica* in India, China, Bangladesh, Sri Lanka etc. The phytochemical screening analysis of plant fraction show the presence of several phytochemical constituents, such as, triterpenes, steroids and cardiac glycosides in the lipophilic fraction and phenolic compounds, flavonoids, alkaloids and saponins in the hydrophilic fraction which may attribute to the medicinal value of this plant [7]. The objective of this study is to perform phytochemical investigation, and to evaluate antioxidant activity of *I. aquatica* from Ghodaghodi wet land area of Kailali district and to make a foundation of further research on this plant.

An antioxidant is any substance that significantly delays or inhibits the oxidation of oxidizable substrates. The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals [8]. Our body is constantly producing free radicals due to regular use of the oxygen which can cause oxidative stress and contribute to various kinds of debilitating health problems. In recent years, substantial evidence has developed supporting a pivotal role for free radicals in many fundamental cellular reactions; suggesting oxidative stress might be cause of common diseases such as atherosclerosis, chronic renal failure, cardiovascular disorders, cancer, aging and diabetes mellitus [9-10]. Antioxidants manufactured synthetically, are called synthetic antioxidants. Some examples of synthetic antioxidants are propyl gallate, butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) etc. Plant products such as fruits, vegetables, and spices are potential sources for natural antioxidants like ascorbic acid, phenolics, carotenoids, tocopherols, flavonoids and folic acid to prevent oxidation [10-11]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay can be used for the estimation of antioxidant potential of natural products of plants. DPPH free radical scavenging mechanism involves generation of a free radical to measure an antioxidant ability of a sample. This method is direct and reliable for evaluating scavenging activity of free radicals. DPPH molecule is a stable free radical because its free electron delocalized over the whole molecule and do not dimerize like other types of free radicals. The characteristic deep violet colour is produced which can be identified by formation of absorption band at 520 nm after addition of DPPH solution to sample solution (hydrogen atom donor). The violet colour of DPPH changes to yellow because of reduction of 2,2-diphenyl-1- picrylhydrazyl radical into 2,2-diphenyl-1-picrylhydrazine (reduced form).The yellow colour left is due to picryl group. The decreased quantity of DPPH molecules can be related with the available hydroxyl ions present on phenolic compounds [12].

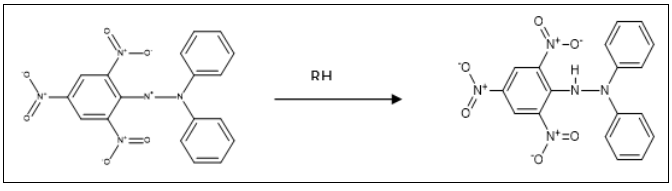

Fig 1: Mechanism of DPPH free radical scavenging

Ascorbic acid, a natural antioxidant, is used as standard in DPPH radical scavenging method.

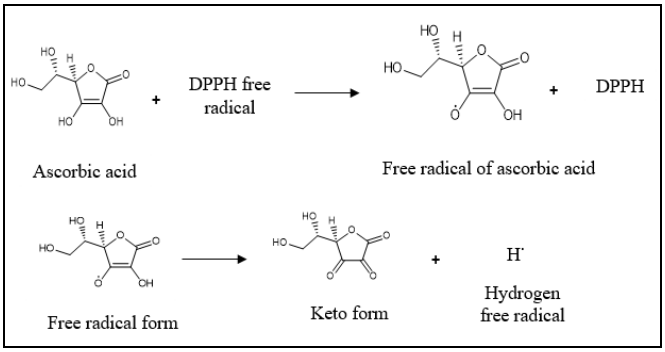

Fig 2: Mechanism of DPPH free radical scavenging by Ascorbic acid

2. Materials and methods

2.1 Chemicals and Equipments

All of the chemicals and solvents used were of analytical reagent grade. The various kind of equipments used were: electrical grinder, weighing balance, hot air oven, rotary evaporator with water bath, UV-spectrophotometer, micro pipettes, glass wares, plastic cuvettes, etc.

2.2 Collection of plants and extraction

The whole plants were collected and washed thoroughly in tap water, cut into small pieces and shade dried and later on it was crushed to get powder. 80 g of powdered sample was weighed out by digital balance and extracted by cold percolation method using hexane and methanol as solvent. Then the filtered extracts were concentrated by using rotary evaporator. The concentrated extract was then kept in a freeze for further analysis [13]. The dried extract was analysed for % yield which was by calculated using the following formula:

$$\text{The percentage yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of the sample}} \times 100\ \% \quad (1)$$

2.3 Phytochemical analysis

Phytochemical analyses of crude extracts of *I. aquatica* were performed based on the procedure described on standard protocol [14-15].

2.4 Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The antioxidant activity of extract was determined using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging assay. 0.0079 g of DPPH was weighed and dissolved in methanol in 100 mL volumetric flask, and final volume was made up to the mark to make 0.2mM DPPH solution. 10 mg of extracts (hexane and methanol) were weighed, dissolved in 10 mL methanol to make stock solution of 1000 µg/mL separately. Solutions having concentration 10, 20, 40, 60, 80 and 100 µg/mL were prepared. Ascorbic acid was used as standard antioxidant. 15 mg of ascorbic acid was weighed and it was dissolved in 15 mL methanol to make the stock solution of 1000 µg/mL. Then by serial dilution solutions of concentrations 10, 20, 40, 60, 80 and 100 µg/mL were prepared. 2 mL of ascorbic acid was pipetted out and mixed with 2 mL 0.2 mM DPPH then the mixture was kept in dark for 30 minutes. 2 mL of methanol was mixed with 2 mL 0.2 mM DPPH and it was also kept in dark for controlled reaction. After 30 min absorbance was measured at 517 nm by using uv–visible Spectrophotometer. Then similar

procedures were performed for all concentrations of ascorbic acid. Similar procedure was followed to measure the DPPH radical scavenging activity of extracts of *I. aquatica*. Control sample was prepared containing the same volume reagents without any extract and reference ascorbic acid. Methanol was used as blank. Percentage scavenging of the DPPH free radical was measured using the following equation.

$$\% \text{ of radical scavenging} = \frac{(Ac - As)}{Ac} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

Where,
A_c = absorbance of control (methanol + DPPH solution)
A_s = absorbance of sample
Then % of radical scavenging or the % inhibitions observed were plotted against respective concentrations used and antioxidant activities of *I. aquatica* were expressed in terms of their IC_{50} values [16-17].

3. Results and discussions

3.1 Percentage Yield

The methanol and hexane extracts obtained from 80 g of dry powder of *I. aquatica* was 9 g and 7 g, percentage yield for methanol and hexane extract were 11.25% and 8.75% respectively.

3.2 Phytochemicals screening

The results of phytochemical screening of different extracts of *I. aquatica* were shown in table given below.

Table 1: Phytochemical analysis of *I. aquatic*

S.N	Phytochemicals	Hexane	Methanol
1	Alkaloids	-	+
2	Carbohydrates	-	+
3	Flavonoids	+	+
4	Phenolic compounds	+	+
5	Coumarin	-	-
6	Saponins	-	+
7	Terpenoids	+	+
8	Quinones	-	+
9	Tannin	+	+
10	Glycosides	-	+
11	Protein and amino acids	-	+

(+) represent presence, (-) represent absence

Phytochemical screening of methanol extract of *I. aquatica* showed presence of wide array of natural bioactive chemical constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolics, saponins, quinones, tannins, terpenoids, protein and amino acid etc. However, relatively less amounts of phytochemicals like flavonoids, phenolic compounds, terpenoids, tannins were found to be present in the hexane extract. In the methanol extract coumarins were found to be absent. Similarly, in the hexane extract alkaloids, carbohydrates, glycosides, coumarins, saponins, quinones, protein and amino acid were absent. However coumarins were absent in both extracts of *I. aquatica*.
Previous research study; proximate analysis on *I. aquatica* was carried out using standard methods in which phytochemical screening of green kangkong revealed high concentrations of alkaloids, reducing sugar, soluble carbohydrate, flavonoids, while it contained lower concentrations of steroids, phenols, glycosides, β-carotene, saponins and tannins. Our study on this plant also exhibit similar result, some of the difference in the phytochemicals may occur due to the variation of geological diversity from place to place. The natural endowments of phytochemicals in rich quantity showed this plant has serious pharmacological and therapeutic effects apart from its nutritional essence [18]. Similar result was reported by Das *et al.*, 2018 indicating the presence of flavonoids, saponins, tannins and steroids in the aqueous and methanolic extracts of *I. aquatica* Forsk. While Shamli and Chandra, 2015 have showed the presence of glycosides, flavonoids, phenols, tannins and terpenoids in acetone extract of *I. aquatica* Forsk. Our result showed methanol is better solvent for the extraction of phytochemicals from *I. aquatica* and also found that *I. aquatica* is a rich source of phytochemicals. Preliminary phytochemical screening is usually performed for the identification of generous phytochemicals which may be responsible for the antioxidant and antimicrobial activity of plant extracts (Sagbo *et al.*, 2017). Total 16 carotenoids were identified of which lutein was major carotenoid and other were β-carotene, violaxanthin, neoxanthin a, neoxanthin b, antheraxanthin, mutatoxanthin, cryptoxanthin**,** lutein epoxide, zeaxanthin, flavoxanthin, auroxanthin, etc from *I. aquatica*. Polyphenols such as myricetin, quercetin, luteolin, apigenin, and kaempferol were also detected in *I. aquatica* [2].

3.3 Antioxidant activity

The antioxidant activity of methanolic and hexane extract of *I. aquatica* was measured by performing DPPH assay. The % of free radical scavenged is monitored by measuring absorbance at different concentration and IC_{50} value of respective extract were calculated from the plot of % inhibition of DPPH vs. concentration.

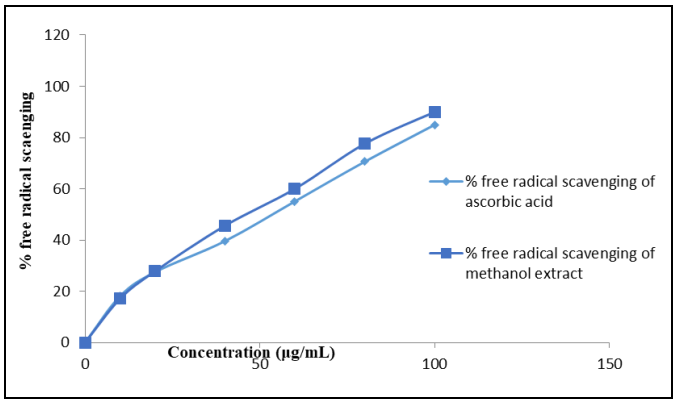

Fig 3: % free radical scavenging activity of Ascorbic acid and Methanol extract of *I. aquatic*

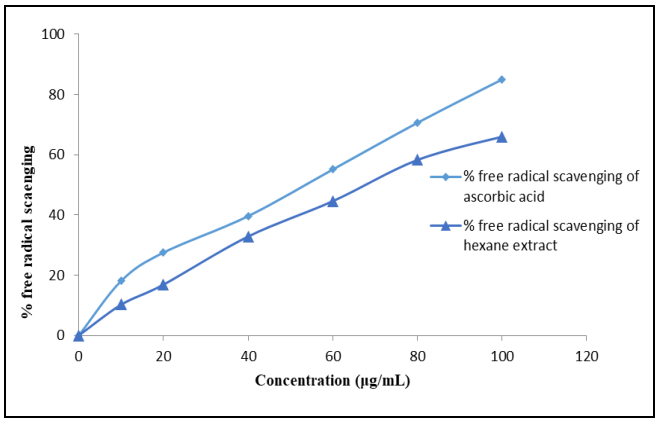

Fig 4: % free radical scavenging activity of Ascorbic acid and hexane extract of *I. aquatic*

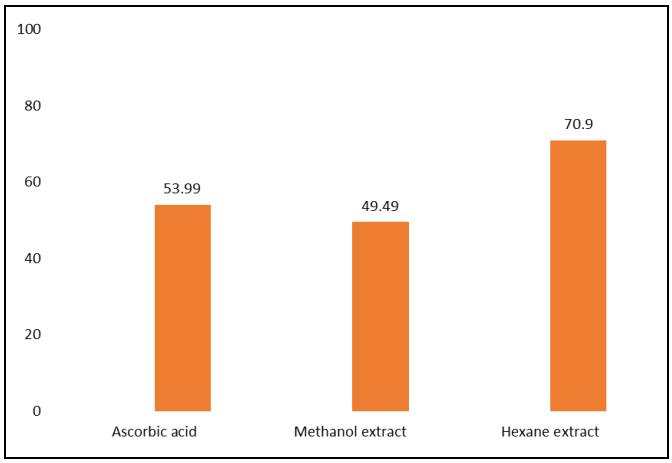

Fig 5: IC_{50} value for ascorbic acid and different extract of *I. aquatica*

The results of antioxidant activities of *I. aquatica* extracts in this study were as shown in Figure 2 expressed in terms of IC_{50} values. IC_{50} value of the extract is defined as the minimum amount of that extract required to reduce the 50% of DPPH free radicals present in reaction solution. The relation between IC_{50} value and the antioxidant capacity of the plant extract is inversely proportional. IC_{50} value of methanol and hexane extracts were calculated and compared with IC_{50} value of ascorbic acid to determine their potential antioxidant activity.

The IC_{50} value for methanol and hexane extract of *I. aquatica* was 49.49 µg/mL and 70.90 µg/mL respectively. Since IC_{50} value of both extract of plant is lower than 100 µg/mL and comparable with IC_{50} value of standard; ascorbic acid (53.99 µg/mL), indicating *I. aquatica* as a rich source of natural antioxidants. For both hexane and methanol extracts of *I. aquatica* antioxidant activity was seen to increase in dose dependent manner. For methanolic extract of *I. aquatica* IC_{50} value is lower than that of ascorbic acid (53.99 µg/mL) which may be due to synergestic effect of phenolic and polyphenolic compounds, flavonoids, proteins, wide array of vitamins etc present in the green leafy *I. aquatica.* Among these two extracts of *I. aquatica* methanolic extract of *I. aquatica* showed higher antioxidant activity than that of hexane extract which may be due to presence of large amount of phytochemicals in later ones.

The methanol extracts of the leaves of *I. aquatica* from western part of Nepal showed potent free radical scavenging activity with IC_{50} =42.43 µg/mL in DPPH free radical scavenging assay. This result is in close agreement with our present research [6]. The methanol extracts of the leaves of *I. aquatica* showed potent free radical scavenging activity with IC_{50} =4.4 µg/mL DPPH free radical scavenging assay. Ascorbic acid was used as standard with IC_{50} value of 15.83 µg/ ml [12].

Similar comparative study for leaves and stem was done by James Omale *et al.* (2011) where IC_{50} values of ethanol extracts were 33.188 µg/mL and 672.376 µg/mL for stem and leaf respectively. In DPPH scavenging assay, the reference standard i.e. vitamin C (IC_{50} = 0.0660 µg/mL) had significantly ($p<0.05$) higher scavenging activity than the stem (IC_{50} = 35.96 µg/mL) which in turn is significantly higher ($p<0.05$) than the leaves (IC_{50} = 176.92µg/ml). From the results it can be concluded that stem and leaves of *I. aquatica* had similar antioxidants activity [19]. Lawal *et al.* in 2016 isolated 3 compounds from hexane and methanol fraction of *I. aquatica* extracts and performed DPPH scavenging assay for these isolated compounds; these compounds show significant antioxidant effect on DPPH free radical [20]. This variation in antioxidant activities may be attributed to different geographical distribution of plants as well as climatic condition based on which secondary metabolites in plants also differ [21-22]. Another research conducted proved that water spinach has higher antioxidant activity than land spinach and there was a correlation between antioxidant activity and total phenol/flavonoids content. Based on TLC results, it was supposed that the compound responsible for antioxidant activities in water spinach and land spinach was flavonoids [23].

4. Conclusions and recommendations

The phytochemical screening of the methanolic extract of *I. aquatica* revealed the presence of alkaloids, carbohydrates, reducing sugars, glycosides, tannins, flavonoids, phenolic compounds. The maximum classes of phytoconstituents are present in methanolic extract as compared to hexane extracts. Hence, the above plant extract could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments. Since this plant has been used in the treatment of different ailments, the medicinal roles of these plants could be related to such identified bioactive compounds. The quantitative analysis of these phytochemicals and their isolation will be more interesting area of research for further study.

Free radical scavenging activity of the plant extract through the annihilation of the DPPH radical showed that *I. aquatica* potent source of antioxidant with the strongest DPPH radical scavenging activity. The possible mechanism of antioxidant activity is due to the presence of phytoconstituents such as flavonoids and polyphenols present in the methanolic extract of plants. The results indicated that the plant in this study is the potent antioxidant source. This plant might be proposed for impeding toxic oxidation in nutraceuticals or drugs for the treatment of coronary diseases. Further investigation into the isolation and identification of responsible antioxidant components and their mechanism of action is necessary to better understand their ability to control diseases that have a significant impact on drug discovery and medicinal chemistry.

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