PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF REINWARDTIA INDICA FROM SELECTED LOCATION OF UTTRAKHAND

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Abstract

***Reinwardtia indica* commonly known as yellow flax, is a low shrub of family Linaceae locally known as pyoli or basnati in the Garhwal region og Uttrakhand. The plant is well known for its ethanobotanical uses by the locals in the cure of various ailments like paralysis, backache, headache, boils and pimples. But well scientific study has not done on this plant so far. So, the current study focuses on the phytochemical composition and antioxidant activity of the plant. The phytochemical screening and antioxidant activity was carried out on the leaf extract of the plant. Preliminary phytochemical screening revealed the presence of tannins, flavanoids, steroids, terpenoids, alkaloids, saponins and reducing sugars in different extracts. While, the antioxidant activity was tested with aqueous and methanol extracts, revealed a significant total Phenolic content, DPPH and Anion scavenging activity.**

Key words- Antioxidant, DPPH, Phytochemicals, Reinwardtia.

Introduction

The history of herbal medicinal practices has been very old, since time immemorial people are using various plants, herbs and shrubs in the cure of various ailments. Among world's richest floras, India is considered as one of the largest flora, due to its wide range of environments, topology and climates. India constitutes 6% of flowering plants of more than 15000 species of the world. In India, the Himalaya is known to have rich biodiversity with 368 families and 2200 genera of medicinal plants. Among these Linaceae family also shares a part with 6 genera and 220 species, commonly known as Flax family, these are distributed in tropical and subtropical region extending to north and south temperate zones. Growing in foothills of Himalaya *Reinwardtia indica* are the low shrubs locally known as pyoli or basanti belongs to the family Linaceae and the genus *Reinwardtia*. There are only two known species of *Reinwardtia* found native to the Southern and Southeast Asia namely *R. indica* and *R. sinensis*[1]. *R*einwardtia have the medicinal as well as ornamental value. The root paste is applied on headache and backache, also with the fruits of *Piper nigrum* is given to eat in measles and the juice of root is been used for the

treatment of fever scabies, wound and indigestion and the roots are used as abortifacient[2]. The stem paste is applied on wounds, cuts, boils, pimples, carbuncle [3] and the wounds infected by maggots in cattle for treatment [4]. The aerial parts are pounded into paste and applied on cuts to stop bleeding and for mouth wash [1]. The leaves are used in the treatment of paralysis [3]. A yellow dye made from the flowers is used for dyeing clothes and making paints. The extracts of whole plant was evaluated in mild to moderate depression cases, proving its anti-depressant activity thus beneficial in the management of mild to moderate depression[1]. Biologically active compounds in plants have always been of great interest, in recent years interest to evaluate plants possessing antimicrobial activity against various microbes has increased. Therefore to explore the antibacterial potential of various unexplored plants has been choice of work for researchers. A large number of phytochemicals belonging to certain chemical classes found to have inhibitory actions against certain pathogens. So, it may be valuable to record the phytochemical compound data for *R. indica*. In ancient Indian literature, it is mentioned that every plant on earth is useful for human beings, animals and other plants. Within the human body, thousands of chemical reactions are occurring constantly. These biological processes requires oxygen, which produces reactive oxygen species (ROS) as byproducts, these are very active free radicals which tend to attack healthy cells, DNA as well as proteins and fats causing their deterioration. Antioxidants are the compounds that can protect human body against damaging effects of ROS. Generally human body has a certain balance between ROS and antioxidants present in the body. An imbalance between antioxidants and ROS results in oxidative stress, to overcome this body needs antioxidants from outside, there comes the utility of plants producing antioxidants. *R. indica* found to have such antioxidants, and has been investigated. Antioxidants from plant resources are potent and safe because they have no side effects.

Materials and Methods

Plant material collection

The plant sample has been collected from wild, in the month of February from Srinagar Gahrwal and Pauri Garhwal region of Uttarakhand.

Extract Preparation

The leaves of *Reinwardtia indica* was shade dried and powered and then extract was prepared in the following solvents: aqueous, chloroform, ethanol, acetone and methanol

5gm of the powdered sample was submitted to the successive solvents separately with 100ml each of the solvents i.e. water, ethanol, methanol, chloroform, and acetone. Left for about 24 to 48 hrs and then filter the extract with fine filter paper [5].

Phytochemical analysis

Screening of all the extracts carried out as for the following phytochemicals compounds-:

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered; the filtrate was dissolved with potassium mercuric iodide. The solution in the test tube gives yellow color showing the presence of the alkaloids. Same filtrate was treated with the Wagner's reagent (Iodine in potassium iodide). Formation of brown reddish precipitate confirms the presence of the alkaloids.

Detection of Phenols

Extracts were treated with 3-4 drops of the ferric chloride solution. Formation of bluish black color indicates the presence of phenolic compounds.

Detection of Tannins

To the extract, add few drops of 0.1% $FeCl_3$. Formation of blue black colour indicates the presence of tannins.

Detection of Flavonoids

Extracts was treated with a few drops of sodium hydroxide solution. Formation of intense yellow

color which becomes colorless on adding dilute acid indicates presence of flavonol group. Same extracts were treated with a few drops of lead acetate solution. Formation of a yellow color precipitate confirms the presence of flavonoids.

Detection of Terpenoids

Extracts was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green solution indicates the presence of diterpenes. Ten mg of extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of conc. H_2SO_4 formation of reddish violet indicates the presence of triterpenes.

Detection of Amino acids

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates presence of amino acids.

Detection of steroids

One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids**.**

Determination of *In vitro* Antioxidant activity

Determination of Total Phenolic Content (TPC) [6]

The Total Phenolic Content of each extract obtained of each of the plant extract was determined and the phenolic content was expressed as µg/g Gallic acid equivalents. In brief a 100 µl aliquot of the sample was added to 2 ml of 0.2% (w/v) Na_2CO_3 solution. After two minutes of incubation, 100 µl of 500ml/l Follin-Ciocalteu reagent added and the mixture was then allowed to stand for 30 minutes at 25^0C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The Total Phenolic Content (TPC) was determined using the standard Gallic acid calibration curve and was expressed as µg/g Gallic acid equivalents.

Determination of Antioxidant Activity by DPPH Radical Scavenging Method [7]

The extract solution for the DPPH test was prepared by re-dissolving 0.2 g of each of the dried extract in 10 ml of the specific solvent in which the extract was prepared. The concentration of DPPH solution was 0.025 g in 1000 ml of methanol. Two ml of the DPPH solution was mixed with 40 µl of each of the plant extract solution and was transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible Systronics spectrophotometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation: Inhibition%= (Abst=0 min---Abst=30 min)/ Abst=0 min ×100 Where Abst=0 min was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5 mM) dissolved in methanol was used as a standard to convert the inhibition capability of plant extract solution to the Ascorbic acid equivalent. IC_{50} is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Superoxide Anion Radical Scavenging Activity [8]

Superoxide Anion Radical scavenging Activity was measured according to the method with some modifications. The different plant extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using Systronics UV-VIS double beam spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control.

The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

RESULTS

Preliminary phytochemical analysis of *R. indica:*

Tests	Methanol extract	Ethanol extract	Chloroform extract	Acetone extract	Aqueous extract
For Tannins **$FeCl_3$ test**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**
For Flavonoid **Ammonia test** **Lead acetate test**	**+ ve** **+ ve**	**- ve** **- ve**	**+ ve** **+ ve**	**- ve** **- ve**	**- ve** **- ve**
For Steroids **Salkowski test**	**+ ve**	**-ve**	**-ve**	**-ve**	**-ve**
For Triterpenoids **Salkowski test**	**+ve**	**-ve**	**+ve**	**-ve**	**-ve**
For Alkaloid **Mayer's test**	**+ve**	**-ve**	**-ve**	**+ve**	**-ve**
For Saponins **Froth test** **Foam test**	**+ve** **+ve**	**+ve** **+ve**	**+ve** **+ve**	**+ve** **+ve**	**+ve** **+ve**
For Phenols **$FeCl_3$ test**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**

Table I: Phytochemical screening Results

The table given above shows phytochemicals present in *R. indica* are tannins, saponins, flavonoids, alkaloids and steroids. Tannins were present in methanolic, ethanolic and water extracts. Flavonoids were present in methanol, ethanol, water and acetone extracts. Alkaloids were present in ethanol, methanol, chloroform and aqueous extracts.

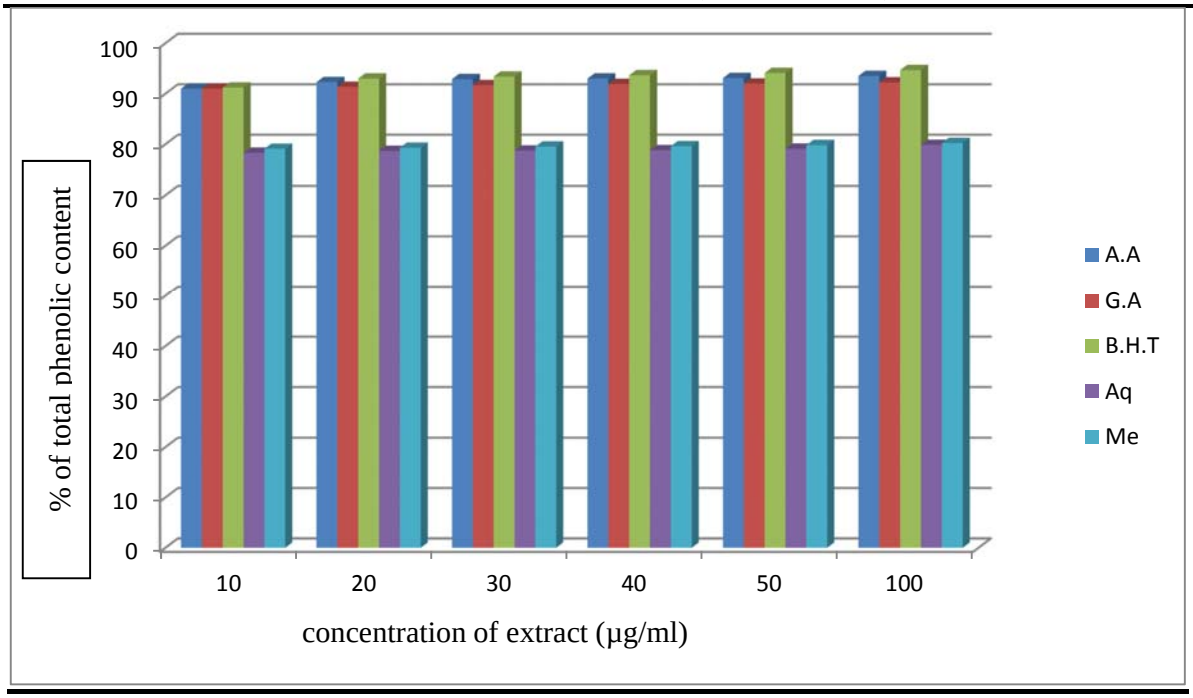
Antioxidant activity:

Total phenolic Content in plant leaf extract:

The assessment of antioxidant activity has been done by using different in-vitro methods. The total phenolic content in Aqueous and Methanolic extracts is shown in Table II, Graph 1. Results showed that the methanol extract of plant exhibited the highest phenolic content(80.25%) followed by aqueous extract of plant(79.87%) and the total phenolic content present in ascorbic acid (93.52%), gallic acid(92.22%), butylated hydroxyl toluene(94.67%).

S.NO	Sample	% of total phenolic content according to concentration of extract (µg/ml)					
		10	20	30	40	50	100
1.	A.A	91.00	92.30	92.88	93.00	93.10	93.52
2.	G.A	91.00	91.35	91.70	91.93	92.00	92.22
3.	B.H.T	91.20	93.01	93.40	93.65	94.10	94.67
4.	Aq	78.30	78.65	78.72	78.80	79.10	79.87
5.	Me	79.09	79.30	79.52	79.60	79.82	**80.25**

Table II: Total phenolic content present in leave extract of *Reinwardtia indica*



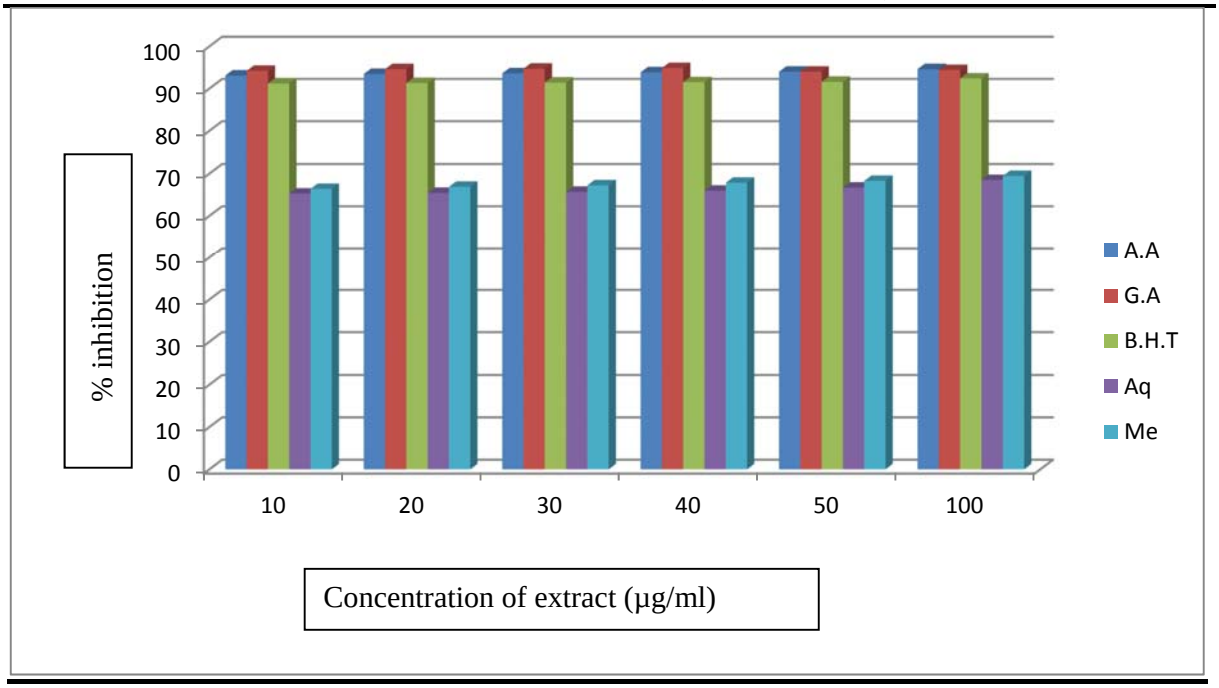
Graph 1: Total phenolic content present in leave extract of *Reinwardtia indica*
A.A: Ascorbic Acid, G.A: Gallic Acid, B.H.T: Butylated Hydroxyl Toluene

DPPH activity:

The free radical (DPPH) activity shown in Table (III), Graph (2), revealed that the methanol fraction of plant exhibited the highest radical scavenging activity with 70.10% followed by its aqueous extract with 69.54% .Methanol has been proven as effective solvent to extract phenolic compounds. Radical scavenging activity present in ascorbic acid (93.52%), gallic acid (92.22%), butylated hydroxyl toluene (94.67%).

S.No	Sample	% inhibition according to concentration of extract (µg/ml)					
		10	20	30	40	50	100
1.	A.A	91.00	92.30	92.88	93.00	93.10	93.52
2.	G.A	91.00	91.35	91.70	91.93	92.00	92.22
3.	B.H.T	91.20	93.01	93.40	93.65	94.10	94.67
4.	Aq	68.03	68.54	68.77	68.83	69.20	69.54
5.	Me	69.10	69.30	69.58	69.80	69.92	**70.10**

Table III: DPPH activities in leave extract of *Reinwardtia indica*

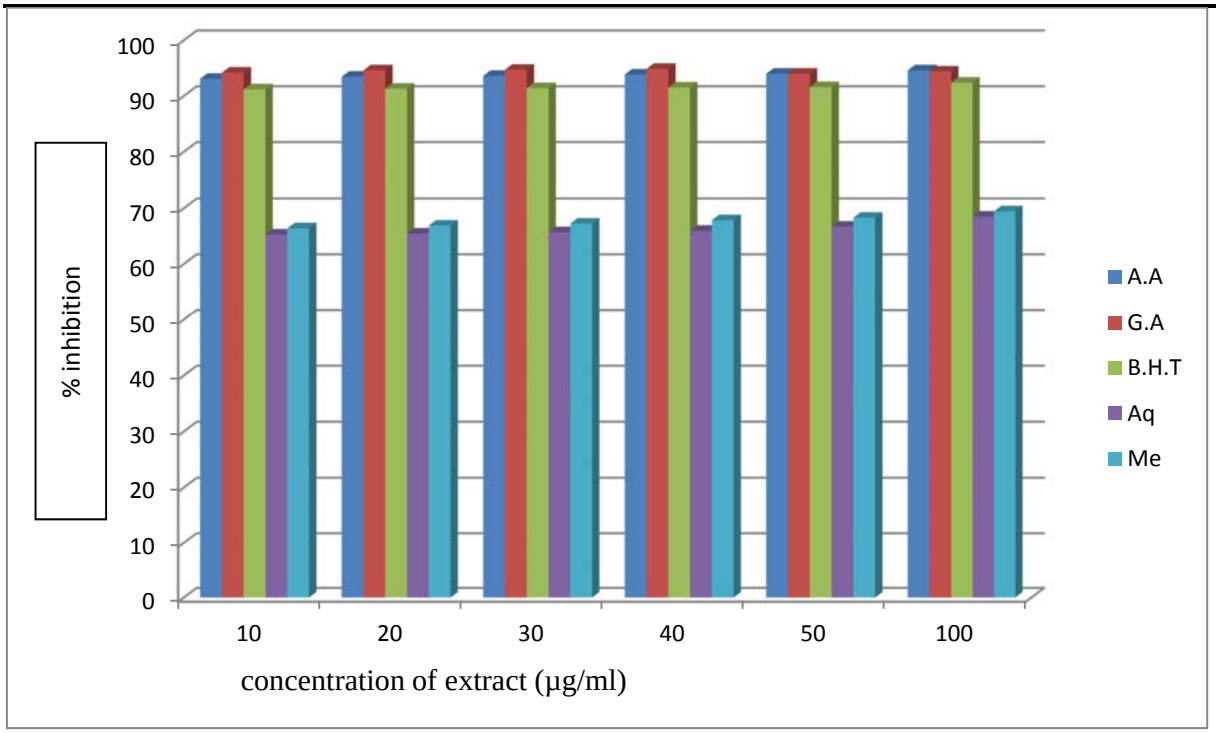


Graph 2: DPPH activities in leave extract of *Reinwardtia indica*
A.A: Ascorbic Acid, G.A: Gallic Acid, B.H.T: Butylated Hydroxyl Toluene, Aq: Aqueous extract of leaves, Me: methanol extract of leaves

Superoxide anion radical scavenging activity: The superoxide anion radical scavenging activity shown in Table IV, Graph 3 shows that the methanol extract of plant exhibited the highest superoxide anion radical scavenging activity with 69.51% followed by its aqueos extract with 68.45%. The superoxide anion radical scavenging activity present in ascorbic acid (94.60%), gallic acid (94.40%), butylated hydroxyl toluene (92.40%).

S.No	**Sample**	**% inhibition according to concentration of extract (µg/ml)**					
		10	20	30	40	50	100
1.	A.A	93.10	93.46	93.60	93.86	94.00	94.60
2.	G.A	94.20	94.61	94.72	94.87	94.00	94.40
3.	B.H.T	91.20	91.31	91.43	91.52	91.60	92.40
4.	Aq	65.30	65.46	65.70	65.97	66.70	68.51
5.	Me	66.40	66.90	67.25	67.86	68.30	**69.45**

Table IV: Superoxide anion radical scavenging activity



Graph 3: Superoxide anion radical sc avenging activity

A.A: Ascorbic Acid, G.A: Gallic Acid, B.H.T: Butylated Hydroxyl Toluene, Aq: Aqueous extract of leaves, Me: methanol extract of leaves

DISCUSSION

The medicinal value of the plant lies in bioactive phytochemical constituents that produce definite physiological action on the human body [3]. Phytochemicals are non-nutritive plant chemicals that have disease preventive properties. Plants have been used since time immemorial for their medicinal value, shown by the various secondary metabolites or phytochemicals synthesized and deposited in specific parts or all parts of them.

The preliminary phytochemical investigation revealed the presence of various constituents of this plant. Different solvent showed different class of phytochemicals. These bioactive phytochemicals are the basis of therapeutic potential of medicinal plants and useful in the treatment of several diseases [6].The phytochemical screening of methanol, ethanol, chloroform, acetone and aqueous extracts of *R. indica* showed a moderate presence of bioactive components such as alkaloids, tannins, flavonoids, saponin, terpenoids and phenolic compounds. Tannins, phenols and saponins are present in all the extracts. Flavonoid showed their presence in methanol and chloroform extract while did not show their presence in ethanol, acetone and aqueous extract. Steroids only showed their presence in methanol extract. Terpenoids showed their presence in methanol and chloroform extract. Alkaloids are present only in methanol and acetone. Due to the presence of these phytochemicals mainly due to alkaloids and saponins the plant extract shows the antimicrobial activity against disease causing bacteria.

Most of the bioactive components have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. An antioxidant is a molecule that slows or prevents the oxidation of the molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result antioxidants are often considered as

reducing agents such as thiols, ascorbic acid and polyphenols [2]. Plant secondary metabolites such as flavonoids and terpenoids play an important role in defense against free radicals [11], Studies have shown that phenolic compounds possess high antioxidant activity and certain therapeutic properties, including anti-diabetic and anti-hypertension activity [12]. Many botanical and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds. The importance of the antioxidant constituents of plant material is to maintain the health and protection from heart diseases and cancer [13].They are vital substances that possess the ability to protect the body from damage caused by free radicals induced oxidative stress [14]. R. indica has been used by the locals in Garhwal Himalaya in the treatment of various ailments which suggests about its medicinal efficacy but the plant has not been studied and explored under modern medicinal science, there is very less or no scientific record found about its medicinal value. The active chemical constituent of the plant is not yet isolated which could help the use of plant against various diseases. Compounds of plant origin are getting preference in Pharma industry due to their specific action and no side effect character. Hence, the detailed study on this plant may leads to the invention of any new drug as well as cure for many diseases.

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