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Preliminary Phyto-chemical Analysis and Antioxidant Activities of Methanol Extract of *Argyreia roxburghii* Choisy

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ABSTRACT
Argyreia roxburghii Choisy is a plant of Convolvulaceae family. The local healers traditionally use the root extract of *Argyreia roxburghii* Choisy especially in epilepsy and in senselessness, because of which the plant was selected for investigation. It has also been used as anti-spasmodic by the village practitioners. The preliminary phytochemical screening of the plant revealed the presence of tannins, saponins, reducing sugars, phenols, glycosides and flavonoid. The antioxidant activity of the methanol extract of *Argyreia roxburghii* Choisy was (MEAR) evaluated by using a range of in-vitro assays. The IC_{50} values were found to be 2.29, 3.91, 6.11 µg/ml in DPPH radical scavenging, nitric oxide scavenging and hydroxyl radical scavenging activity. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic (13.38 µg/ml Gallic acid equivalent) and flavonoid (1.02 µg/ml quercetin equivalent) components. The result obtained in the present study indicates that the *Argyreia roxburghii* Choisy root is a potential source of natural antioxidant.

Keywords: *Argyreia roxburghii* Choisy, MEAR, Antioxidant, Flavonoid, Total phenol content.

1. Introduction
Argyreia is one of the largest genera of Convolvulaceae family. It contains about 90 species and is distributed in tropical continental Asia, Malaysia and Australia. Most plants are woody climbers with large showy flowers. *Argyreia roxburghii* Choisy is collected from local area of Dibrugarh, Assam, India is a plant of Convolvulaceae family. It is known by three different local names, the Sonborial, Hemlata, and Sonparua lata in Assam. The local healers traditionally use the root extract of *Argyreia roxburghii* Choisy especially in epilepsy and in senselessness, because of which the plant was selected for investigation. It has also been used as anti-spasmodic [1, 2] by the village practitioners. Literature does not reveal any phytochemical or relevant works done on this plant. In order to value the pharmacological activities of *Argyreia roxburghii* Choisy, we were interested in the preliminary study in the extraction and the characterization of the active principle. The objective of the present study was phytochemical screening and to investigate antioxidant activity of the methanol extract of *Argyreia roxburghii* Choisy (MEAR) root in different *in-vitro* studies as well as determination of total phenol and flavonoid content in order to evaluate the relationship between the antioxidant activity and the phytochemical constituents.

2. Materials and Methods
2.1 Collection of samples
The fresh roots of *Argyreia roxburghii* Choisy was collected during the month of April from local areas of Dibrugarh district, Assam, India. The identity of the plant was confirmed at the Botanical Survey of India, Office of the Joint Director, Eastern Circle, Shillong, India. The voucher specimen is being preserved in the department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

2.2 Chemicals
1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from- Sigma-Aldrich Chemie Gmbh Germany-, sodium nitroprusside; napthyl ethylenediamine dihydrochloride (NEDD) from Himedia and all other chemicals were purchased from Loba Chimie and Merck, Mumbai, India.

2.3 Preparation of Extract
Methanol extract of the root were used in the present study. The roots were washed clean with distilled water, drain dried and cut into small pieces and dried in shade and ground into fine powder in a mechanical grinder and stored in an air tight container. The plant material was first defatted with pet-ether (60-80) then extracted with methanol using soxhlet apparatus. The extract was first concentrated in a rotary vacuum evaporator (Buchi) and the reddish brown sticky mass obtained was used for the tests.

3. Experimental procedures
3.1 Preliminary phytochemical screening
The preliminary phytochemical screening of the root powder and the methanol extract of *Argyreia roxburghii* Choisy was performed qualitatively for the presence of tannins, saponins, reducing sugars, glycosides, phenolics, flavonoids, alkaloids, terpenoids and steroids according to the standard methods which revealed the presence of tannins, saponins, reducing sugars, phenols, glycosides and flavonoids [3-5].

3.2 *In-vitro* antioxidant activity
3.2.1 DPPH radical scavenging activity
DPPH radical scavenging activity was measured using the method of Naskar *et al* [6-9]. With some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (0.002% in methanol) and 1 ml of test solution at various concentrations (4, 8, 12, 16 µg/ml) of the methanol extract of *Argyreia roxburghii* Choisy was incubated at 37 ºC for 30 min and absorbance of the resulting solution was measured at 517 nm using Varian model Cary 100 spectrophotometer. Ascorbic acid was used as standard compound for comparison. The amount of the extract in ug/ml at which the absorbance decreased to half its initial value was taken as IC_{50} value for the extract. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract or the standard) using the following equation:

Percentage Inhibition = (1- absorbance of the test / absorbance of the control) x 100 (Eq.1)

3.2.2 Nitric oxide scavenging activity
The nitric oxide radical scavenging activity of MEAR was assayed according to the method of Naskar *et al*. (2010). Nitric oxide was generated from sodium nitroprusside and nitrite formed was measured by the Griess reaction. The nitric oxide generated by sodium nitroprusside in aqueous solution at the physiological pH interacts with oxygen to produce nitrite ion [6, 7, 10-12] which can be measured by the Griess reaction. About 1 ml of the extract solution at different concentrations (4, 8, 12, 16 µg/ml) in phosphate buffer (pH 7.4) was mixed with 1 ml of 10 mM sodium nitroprusside and the mixture was incubated at 25 ºC for 150 min. From the incubated mixture, 1ml was taken out and 1ml of the Griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylenediamine dihydrochloride), was added to it. Then absorbance of the chromophore so formed was measured at 546 nm using Varian model Cary 100 spectrophotometer. Rutin was used as standard compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of the test with those of the control using (Eq. 1).

3.2.3 Hydroxyl radical scavenging activity
The scavenging capacity for hydroxyl radical of *Argyreia roxburghii* Choisy was assayed according to the modified Fenton reaction of Olabinri *et al*. (2010) [11-15]. Hydroxyl radical was generated in vitro by mixing iron(II) sulphate with hydrogen peroxide and 1,10- phenanthroline. The 1, 10-phenanthroline was used as a redox indicator. The H_2O_2 / Fe^{2+} system produces hydroxyl radical through the Fenton reaction. The hydroxyl radical produced was determined due to change in absorbance at 560 nm. The reaction mixture contained 60µl of 1.0 mM $FeSO_4.7H_2O$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8) 150 µl of 0.17 M H_2O_2 and 1.5 ml of the extract at different concentrations (10, 20, 30, 40, 50 µg/ml). After incubation at room temperature for 5 min, the absorbance of the mixture was measured at 560 nm using Varian model Cary 100 spectrophotometer. Ascorbic acid was used as standard compound for comparison. The IC_{50} defined as the concentration of sample at which 50% of hydroxyl radical was scavenged and calculated for each sample. The hydroxyl radicals scavenging activity was calculated according to (Eq. 1).

3.2.4 Reducing Power
The ferric reducing power of the MEAR was determined on the basis of the ability of their reducing potential by the method reported by Naskar *et al*. [6, 8, 12, 13, 17]. 1ml of different concentrations (4, 8, 12, 16 µg/ml) of the test solution were mixed with potassium ferricyanide (2.5 ml 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50 º for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out and to this 2.5 ml de-ionized water, 0.5 ml $FeCl_3$ (0.1%) were added. Absorbance was measured at 700 nm and compared with ascorbic acid. Higher absorbance of the reaction mixture indicated higher reducing power.

3.3 Determination of total phenol content
The amount of total phenol content of the extract MEAR was determined by the modified method of Amin Ardestani *et al* [6, 8]. Exactly 0.5 ml of the extract was transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by addition of distilled water. A 1 ml of the Folin-Ciacalteu reactive solution was added to the solution and incubated for 3 min at room temperature. Then 3 ml of 2% solution of Na_2CO_3 was added and the mixture was shaken for 2 h. at room temperature. The absorbance was measured at 760 nm. Gallic acid was used for calibration curve and the amount was expressed as Gallic acid equivalent. The concentration of total phenol compounds in MEAR was determined as microgram per ml.

3.4 Determination of total flavonoid content
The total flavonoid content of MEAR was determined by a colorimetric method as described by Amin Ardestani et al [6, 8] in the literature. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%). After 6 min, 0.15 ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, deionized water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then

determined at 415 nm. Results were expressed as quercetin equivalents (µg quercetin /ml extract).

4. Statistical analysis

Experimental analyses were performed in triplicate. Data were recorded as mean ± SEM and analyzed by Graph pad prism. Statistical significance was considered at $p< 0.05$. Linear regression analysis was used to calculate IC_{50} values.

5. Results and Discussion

5.1 Preliminary phytochemical screening

The summary of the preliminary phytochemical studies on the methanol extract of *Argyreia roxburghii* Choisy are shown in Table 1. The sign (+) indicates the presence of the constituent while (-) indicates the absence of the constituent. The screening revealed the presence of tannins, saponins, reducing sugars, glycosides, phenolics and flavonoids.

Table 1: Result of the phytochemical screening on the methanol extract of *Argyreia roxburghii* Choisy root.

Constituents analyzed	Remarks
Tannins	+++
Saponins	+++
Glycosides	++
Phenolics	++
Flavonoids	+
Reducing sugars	+++
Alkaloids	-
Terpenoids	-
Steroids	-

5.2 *In-vitro* antioxidant activity

In-vitro antioxidant activities of the MEAR root has been evaluated by using a range of in-vitro assay. The findings of the *in-vitro* studies are expressed in the form of median inhibitory concentration (IC_{50}) are shown in Table 2.

Table 2: The IC_{50} values, total phenol content and total flavonoid content in the methanol extract of *Argyreia roxburghii* Choisy root.

Antioxidant Activity	IC50		
	MEAR	Ascorbic Acid	Rutin
DPPH Radical Scavenging Activity	2.29	3.36	-
Hydroxyl Radical Scavenging Activity	3.91	6.49	-
Nitric Oxide Scavenging Activity	6.11	-	13.73
Total phenol content	13.38 µg/ml	-	-
Total flavonoid content	1.02µg /ml	-	-

5.2.1 DPPH radical scavenging activity

Free radical scavenging potentials of MEAR at different concentrations were tested by the DPPH method and the results are shown in Figures 1.

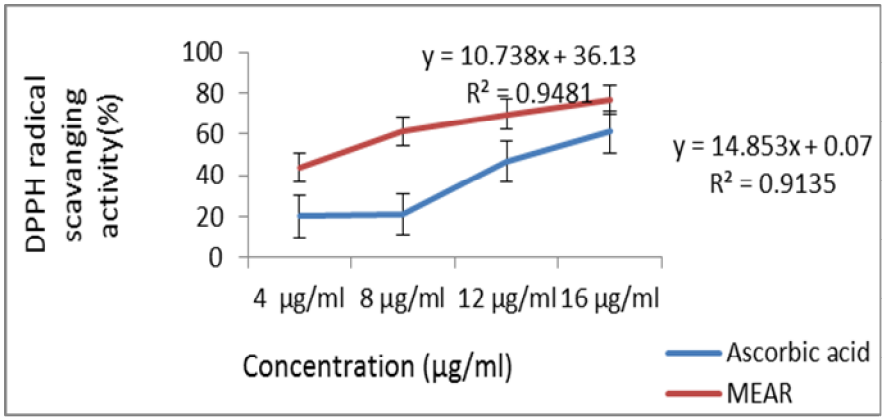

Fig 1: Determination of DPPH radical scavenging activity of MEAR

Antioxidant reacts with DPPH and the degree of discoloration indicates the scavenging potentials of the antioxidant extract. The DPPH radical scavenging activity of MEAR was compared with ascorbic acid. The IC_{50} values for MEAR and ascorbic acid are 2.29 µg/ml and 3.36 µg/ml, respectively according to this method.

5.2.2 Nitric oxide scavenging activity

MEAR effectively reduced the generation of nitric oxide from sodium nitroprusside. The extract showed nitric oxide scavenging activity (IC_{50} 6.11 µg/ml) that of standard rutin was (IC_{50} 13.73 µg /ml). MEAR appreciably decreases the amount of nitrite generated from the decomposition of sodium nitroprusside *in-vitro.*

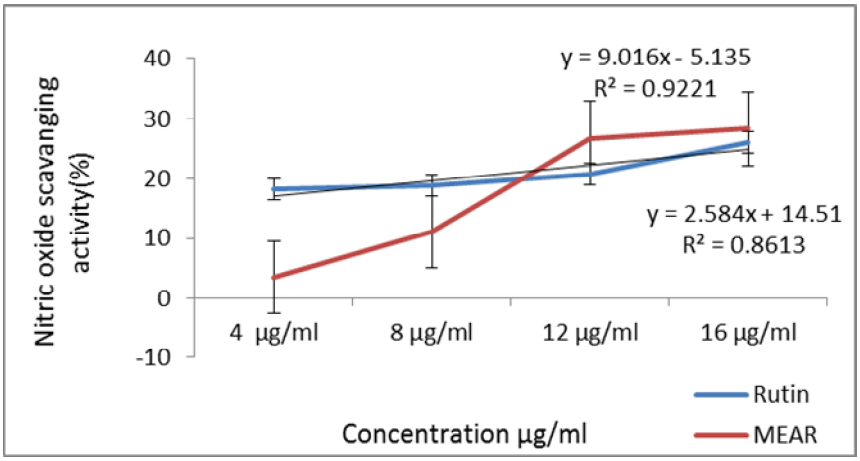

Fig 2: Determination of nitric oxide radical scavenging activity of MEAR

5.2.3 Hydroxyl radical scavenging activity

Hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism among the reactive oxygen species, which could be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron and cause the aging of human body and some diseases (Siddhuraju and Becker, 2007). Hydroxyl radical formation can occur in several ways; the most important mechanism *in vitro* is the Fenton reaction where a transition metal is involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide (Jin and Wang, 2011). Figure 3 indicates that the MEAR exhibited a low inhibition of hydroxyl radical compared to Ascorbic acid. The IC_{50} of MEAR was only 3.91 µg/ml and the IC_{50} of ascorbic acid was 6.49 µg/ml.

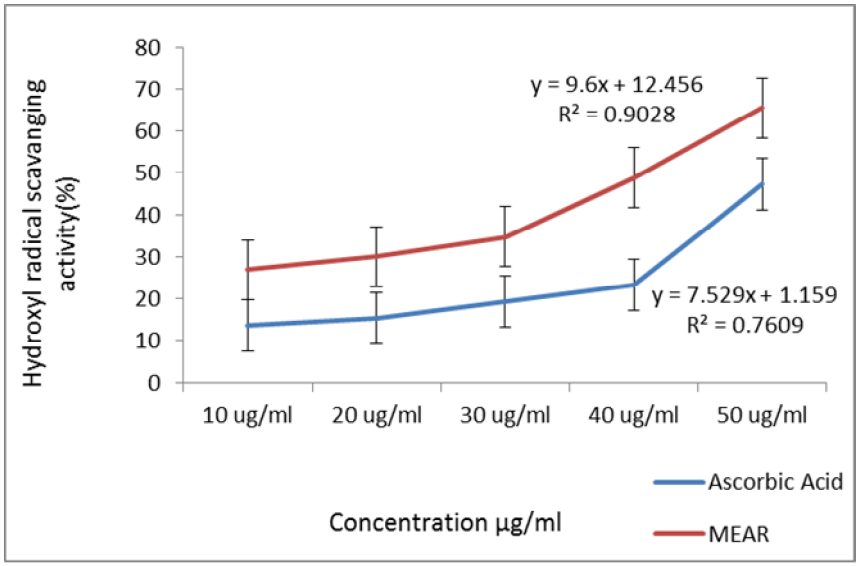

Fig 3: Determination of hydroxyl radical scavenging activity of MEAR

5.2.4 Reducing Power

Figure 4 shows the reductive capabilities of MEAR when compared to the standard ascorbic acid. Like the antioxidant activity, the reducing power increased with increasing amount of the extract. For the measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was investigated in presence of the extract. The high co-relation co-efficient (R^2=0.927 in figure 4) indicates higher reducing power of the MEAR.

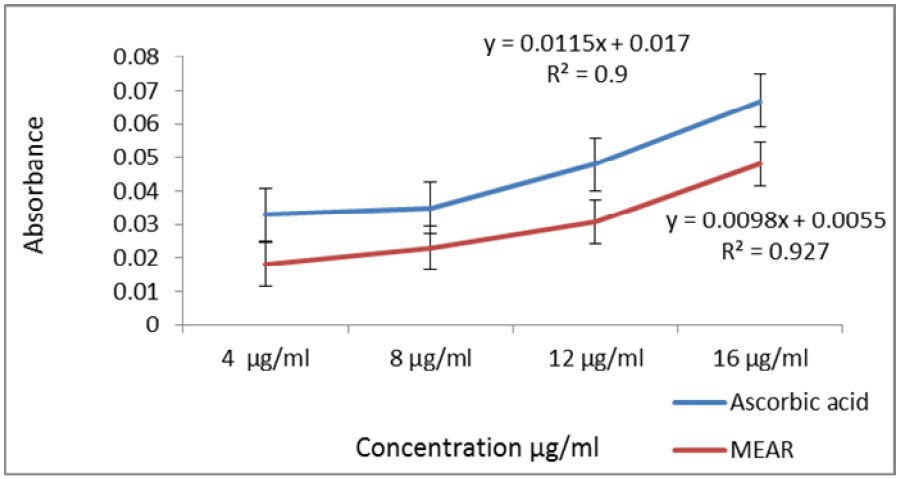

Fig 4: Determination of reducing power of MEAR

5.3 Determination of total phenol content

Figure 5 shows the determination of total phenol compound of MEAR. Total Gallic acid equivalent of MEAR was found to be 13.38 µg/ml.

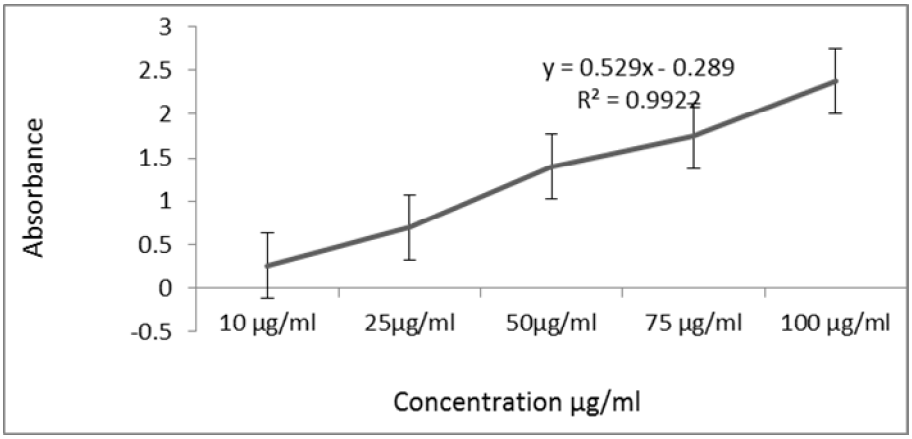

Fig 5: Determination of Total Phenol content of MEAR

5.4 Determination of total flavonoid content

Flavonoids in plants exhibit antioxidant activity. The flavonoid content of MEAR root extract is shown in Fig. 6. Total flavonoid content of MEAR root was found to be 1.02 µg/ml of quercetin equivalent of extract.

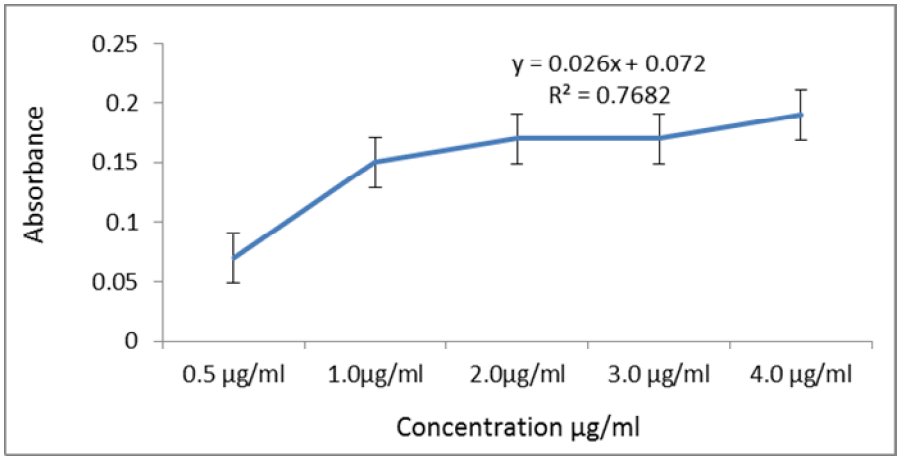

Fig 6: Determination of Total flavonoid content of MEAR

The phytochemical screening of the crude MEAR reveals the presence of phenolics, saponins, tannins and flavonoids. The antioxidant activity of methanol extract of *Argyreia roxburghii* Choisy (MEAR) was evaluated by using four different methods, namely the DPPH radical scavenging, nitric oxide radical scavenging, hydroxyl radical scavenging activity and reducing

activity of the MEAR. In all these four methods the MEAR shows appreciable antioxidant and free radical scavenging activities, which may be due to the presence of the Phyto-constituents like phenols in the extract. The total phenol content of MEAR is quite appreciable.

6. Conclusion

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemical to highly reactive species such as free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoid scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The herbal preparations may have such antioxidant activities that can help protecting the cellular damage and stops degeneration which enhance in other biological activities as shown by the plant extract. The present investigation suggests that MEAR shows good in-vitro antioxidant activity and reducing power. The antioxidant activity of *Argyreia roxburghii* Choisy may have contributions on the anti-epileptic activity as practised by the local healers. Further studies regarding the isolation and characterization of active constituents in methanol extract of *Argyreia roxburghii* Choisy and evaluation of their biological activities is currently under progress.

7. Acknowledgment

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