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Phytochemical screening and study of *in vitro* antioxidant activity of two different extracts of *Andrographis paniculata* Nees (Family: Acanthaceae)

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Abstract
Andrographis paniculata Nees is a medicinal plant used traditionally for the treatment of various diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, skin diseases etc. In the present study, methanolic and petroleum ether extracts of the plant sample were taken and screened for the presence of secondary metabolites. The same extracts were again used for the antioxidant assays using DPPH and by estimation of their Total Flavonoid Content (TFC). The presence of most of the secondary metabolites was observed in the methanolic extract along with significant antioxidant activities compared to the petroleum ether extract.

Keywords: DMSO, DPPH, IC_{50}, TFC

1. Introduction
Medicinal plants are being used from time immemorial for their therapeutic values in different countries for their healing properties to heal various diseases and because of the fact that they are cent percent natural. They have been identified to possess numerous phytochemicals with potential biological activity, thereby playing a restorative role in shielding humans from various diseases and complications, which is why they are utilized by a large proportion of the population (Ruwali and Negi, 2019) [1]. The phytochemicals present in the medicinal plants such as carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins and saponins possess various activities that help in scavenging the free radicals [2]. This antioxidant property is of huge importance as it has the potential to suppress the use of synthetic antioxidants [3].
Andrographis paniculata Nees (Acanthaceae), naturally occuring in South India (*kalmegh*) and South East Asia (*chuanxinlian*), is an important ingredient used in herbal traditional Indian and Chinese medicinal [4, 5]. The present study attempts at phytochemical screening and evaluating the *in vitro* antioxidant activity of *A. paniculata* extract, thereby attempting to verify the textual and orally transmitted claims.

2. Material and Methods
2.1 Collection of plant Material
The root bark of *Andrographis paniculata* Nees was collected in July from Sivasagar district (Assam). The plant material was identified by Dr.Pankaj Chetia, Assistant Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh (Assam, India).

2.2 Extraction procedure
The root bark of *Andrographis paniculata* Nees was shade-dried for 3 weeks, mechanically crushed into fine powder and stored in an airtight container for further studies. The powder was introduced into the chamber (extractor) and extracted with methanol and continued till the dark green color of the leaf of the plant became colourless. After extraction, the concentrated solvents of distilled pot are evaporated in rotary evaporator. The same process was done for Petroleum ether extract also.

2.3 Phytochemical Screening
Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the methanolic as well as petroleum ether extracts of root parts of *A. paniculata* [6, 7].

2.4 DPPH free radical scavenging activity
The ability of *A. paniculata* extracts to scavenge the DPPH radicals was assessed by using the method of Blois [8].

2.5 Screening for Total Flavonoid Content (TFC)
The total flavonoids content was estimated using the procedure described by Jia *et al* [9].

3. Results
3.1 Screening of Phytochemical Constituents
The methanolic root bark extract of *Andrographis paniculata* showed the presence of terpenoids, carbohydrates, reducing and non-reducing sugars, alkaloids, flavonoids, tannins, sapnonin glycosides etc. Petroleum ether, on the other hand contains only flavonoids, phenols and fats. Amino acid, anthocyanin and protein were absent in both, petroleum ether and methanolic extract.

Table 1: Results of Phytochemical Analysis of Methanolic and Petroleum ether extract

S. No.	Tests	Observation	
		Petroleum Ether extract	Methanolic extract
1	**Test for carbohydrates**		
	Molish's Test	-	+
2	**Test for reducing sugar**		
	Fehling's Test	-	+
	Benedict's Test	-	+
3	**Test for Non-Reducing Sugar**		
	Iodine Test	-	+
4	**Test for proteins**		
	Biuret Test	-	-
	Xanthoprotein Test	-	-
5	**Test for amino acid**		
	Ninhydrin Test	-	-
	Milon's Test	-	-
6	**Test for fats and oils**		
	Saponification Test	+	+
	Solubility Test	+	+
7	Test for terpenoids	+	+
8	Test for saponin Glycosides (Foam Test)	-	+
9	**Test for flavonoids**		
	Shinoda Test	+	+
	Zinc hydrochloride reaction test	+	+
10	**Test for Tannins and Phenolic compounds**		
	Ferric Chloride Test	+	+
	Lead Acetate Test	+	+
11	**Test for Alkaloids**		
	Dragendroff's Reagent Test	-	+
	Mayer's Reagent Test	-	+
	Hager's Reagent Test	-	+

+: Indicates the presence and -: Indicates the absence of phytoconstituents

3.2 DPPH Free Radical Scavenging Assay
DPPH free radical scavenging assay was carried out for determining the antioxidant activity of the methanolic and petroleum ether extracts of *Andrographis paniculata.* The antioxidant activity was measured in terms of IC_{50} values.

Table 2: Inhibition % of Methanol and Petroleum ether extracts of *A. paniculata*

S. No.	Concentration (µg/ml)	Inhibition%	
		Petroleum ether extract (PEAP)	Methanol extract (MEAP)
1	100	73.25	76.02
2	50	72.64	75.86
3	25	72.48	74.17

The above table shows that the methanolic extracts of *A. paniculata* exhibit maximum DPPH inhibition of 76.02% at 100 µg/ml, while that of petroleum ether exhibited 73.25% inhibition at the same concentration. Also, the IC_{50} value of methanolic extract is less compared to the other. Thus, conclusively methanolic extract exhibits higher antioxidant activity compared to petroleum ether extract of the plant sample.

3.3 Total Flavonoid Content (TFC)
The estimation of Total Flavonoid Content (TFC) was done taking rutin as standard. The antioxidant activity of both the extracts was determined with the Total Flavonoid Content.

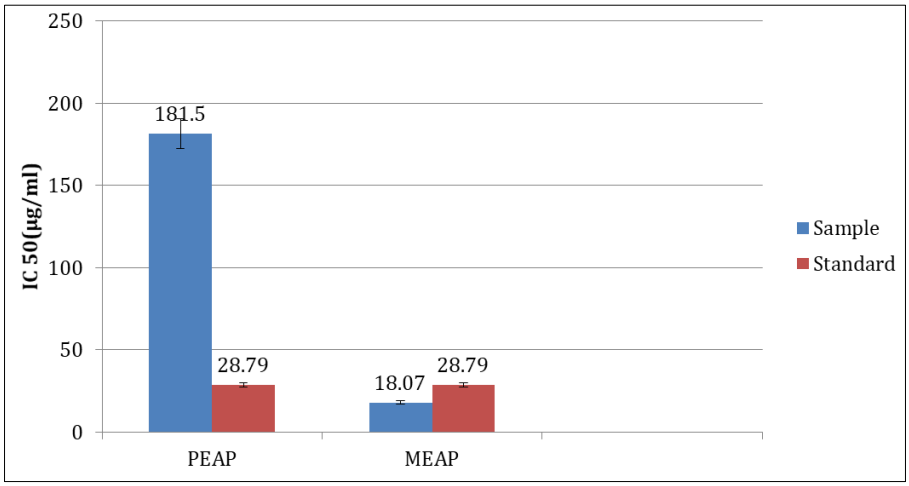

Fig 1: Comparative graphical study of antioxidant activity of plant extracts against Ascorbic acid with their respective IC_{50} values.

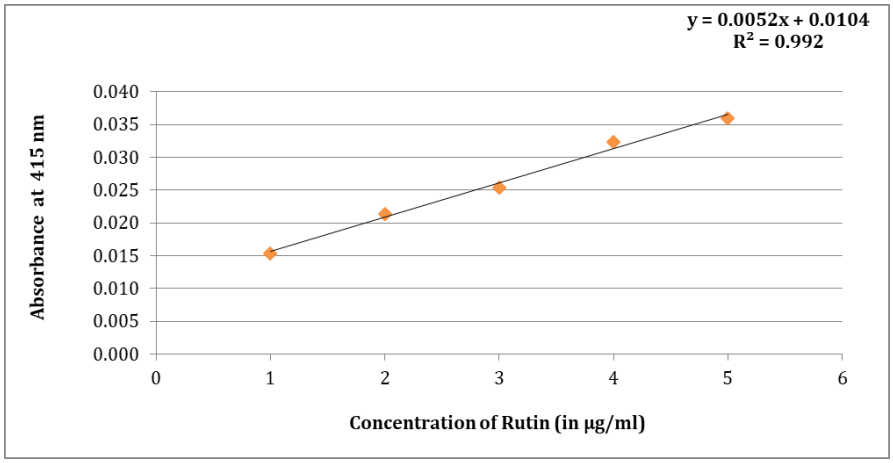

Fig 2: Table showing concentration of standard (Rutin) for estimation of TFC

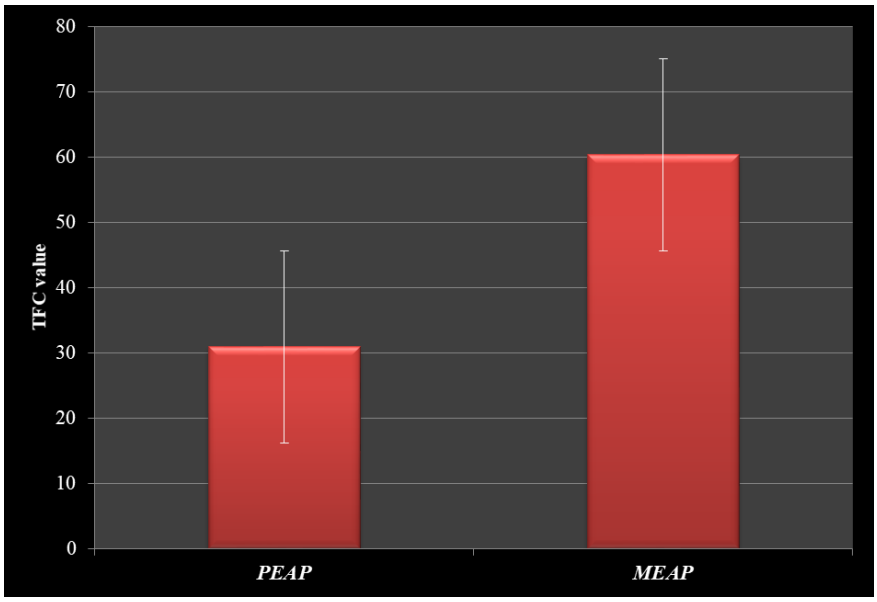

Fig 3: Bar diagram depicting the TFC of both petroleum ether and methanolic extracts of *Andrographis paniculata*

4. Conclusion

Methanolic extract of *Andrographis paniculata* showed the presence of most of the phytochemical constituents *viz.* terpenoids, alkaloids, tannins, flavonoids etc. Regarding antioxidant activity, methanolic extract of *A. paniculata* exhibited better DPPH free radical scavenging activity compared to petroleum ether extract of the sample. The determination of Total Flavonoids Content of results showed that the methanolic part of this extract contain higher amount of flavonoid than petroleum ether extract.

5. References

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