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Phytochemical and proximate composition of petroleum ether, ethanolic and aqueous extracts of leaf, seed, stem and bark of Azadirachta indica

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Abstract

The plants and extracts contain active secondary metabolites responsible for various biological and physiological activities in living beings. Three extraction solvents applied on the ground seeds, leaves, stems and barks part of *Azadirachta indica* were analysed for proximate and phytochemicals using Association of Official Analytical Chemists, AOAC International standard procedures. The results of proximate analysis revealed significantly different ($p<0.05$) ranges of ash content (4.8 – 10.4%), crude fat (10.2 – 12.0%), fibre(12.0 – 15.6%), protein (11.9 – 14.0%) and carbohydrate (53.6 – 57.5%) accordingly. The dried seed kernel contained the highest amounts of crude fat, fibre and protein. However, the leaf and stem samples contained highest ash and carbohydrate contents respectively. The results showed a significant difference for all samples. The phytochemicals observed were saponins, tannis, flavonoids, alkaloids, anthracene and cryogenic glycosides. There was a strong indication of the phytochemical's presence in the leafy and seed samples, where saponins appeared in higher concentrations.
This study has provided a baseline for the applicability of petroleum ether, PET as a better extraction solvent in the derivation of phytochemicals in the plants. Petroleum ether is less hygroscopic and more selective for hydrophobic lipids and organic compounds.

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Introduction

Plants are known to possess beneficial properties in terms of health, nutrition, economy, industry and agriculture (Gul*et al*., 2017; Joshua *et al*., 2016; Salau *et al*., 2015). Healing power of medicinal plants is generally known to man since dawn of civilization which has transformed into present day pharmaceutical skills. These plants are grown all over the globe and contain active components or secondary metabolites which form the precursor or lead compounds in the industry such as tannins, alkaloids, flavonoids, saponins etc. which have been found in-vitro to have medicinal properties (Gul*et al*., 2017; Joshua *et al*., 2016). Researchers have investigated these active components in different plants to produce synthetic versions for pharmaceutical drugs. Amongst these medicinal plants is *A. indica* (neem) plant (Prajapati and Prajapati, 2002; Latif*et al*., 2003; Shinwari*et al*., 2006; Susmitha *et al*., 2013)
Azadarchta indica (neem tree) is Maliceae family, Azadirachta genus and indica species. It is a known in India importantly as a medicinal tree that has lots of biological activity and is called a tree for solving global problems. It is also described as 'Arishthna' in Sanskrit, meaning a reliever of sickness (Siddiqui and Ali, 1997; Biswas *et al*., 2002)
Researchers have shown that *A. indica* plant contains compounds that can control 100 species of insects and microorganisms (Vaideki *et al*., 2007). Also, it is reported that different parts of the plant are individually medicinal against different ailments. There are more than 135 compounds with detailed chemistry and structural diversity isolated from different parts of *A. indica* tree, to include nimbin – a first bitter compound isolated from the plant (Siddiqui and Ali, 1997).
Clinical studies with extracts of *A. indica* can effectively cure chronic and acute ringworm, eczema and scabies within days of therapeutic applications (Susmitha *et al*., 2013). Other therapeutic effects of A. indica which have been administered are against growths of pathogen, bacteria, fungi and viruses (Susmitha *et al*., 2013), free radical (Ghimeray *et al*., 2009), kidney stone (Abdel Monein, 2004) and blood infection (Baligar, 2014) etc. It is noteworthy that *A. 'indica* plant extracts show target sites other than sites used by antibiotics will be active against drug resistant

microbial pathogen (Susmitha *et al*., 2013). The application of various solvents for extraction of phytoconstituents is reported to have produced different yields. This is as a result of action of solvents to extract phytoconstituents in different amounts and properties (Amadi *et al*., 2017). However, little work has been reported on the use of petroleum ether, PET extraction of *A. indica* in quantification of proximate property and phytoconstituents. This work is, therefore, compared the proximate analyses and phytochemicals quantification of *A. indica* leaf, seed, stem, and bark extracts using petroleum ether, ethanol and aqueous as solvents, and to determine which solvent is suitably more selective of secondary metabolites in the plant parts.

Materials and Methods

Collection of the plant materials: The Neem plants, *A. indica* and its parts were collected from within the University of Technology Minna Bosso Campus. The plants were thoroughly washed with water to remove foreign bodies and air dried for days and then oven dried at 105ºC. The samples were ground using a mechanical grinder until finely particles (powder) were obtained. The powdered forms of samples were kept air-tight in containers prior to analysis.

Determination of proximate composition:Proximate analyses of the samples for carbohydrate, energy, ash, crude protein, fiber and fat contents were carried out according to standard methods of AOAC (1990).

Preparation of Sample Extracts: The extraction of *A. indica* plant samples was done with soxhlet apparatus. 5 g of each plant sample was measured into a thimble; 200 ml of solvents (Petroleum Ether, Ethanol and Distilled water) was measured into the flask. The soxhlet apparatus was assembled with the temperature adjusted to 40-60 oC for 6 hrs extractions. Each extract was filtered and decanted. The process was repeated three times, the extracts from each process were pooled and vacuum dried to concentrate the extracts and recover the solvents. The aqueous, petroleum ether and ethanol extracts of *A. indica* were labeled accordingly prior to phytochemical screenings. The screening was carried out in test tubes.

Phytochemical Screenings: Phytochemical screenings were done to find the presence of the active chemical constituents by the standard procedures described by Edeoga *et al.* (2005) and Harborne (1996).

Alkaloids: 0.5 g of each extract was treated with 5 ml of 1% aqueous hydrochloric acid in a water bath and filtered. 1cm of each extract was treated with Meyer's reagent and Hager reagent. A deep brown creamy precipitate was observed and this indicates that the result was positive.

Flavanoids: 1 cm^3 of each extract was dissolved in Sodium hydroxide. The solution turned yellow in colour but disappears on addition of Hydrochloric acid. This indicated the presence of flavanoids.

Saponins: A portion of the extract was treated with distilled water and shaken vigorously which was allowed to stand for about five minutes. Persistent frothing was obtained in some of the extract. This indicates a positive test.

Tannins: A portion of the extract was diluted with distilled water and 2 drops of 10% Ferric chloride was added which gave a green colour.

Cynogenic glycosides: 1.0 g of the extract is covered with sufficient water in a stopered flask into which Sodium picrate paper is colour suspended by trapping it with a cork. The flask is placed in a water bath for 1hr. a change from yellow colour of the paper to brick red colour is a positive result for cynogenic glycosides.

Anthracene glycosides: A mixture of 5.0ml of dilute sulphuric acid and 5.0ml ferric chloride solution is added to 0.5 ml of the extract. The resultant mixture is boiled for 5 minutes, cooled and filtered into a 50 ml separatory funnel. The filtrate is shaken with equal volume of carbon tetrachloride. The lower organic layer is carefully separated into a test tube and 0.5 ml of dilute ammonia solution added to it with gently shaking. Observe for pink colouration in ammonia layer gives a positive test.

Results and Discussion

The proximate composition (%) of dreied sample of aqeous extract of *A. indica* revealed the present of ash, crude fat crude fibre, crude protein and carbohydrates (Table 1)

Table 1 Mean proximate analysis (%) of dried samples

CONTENT	% Seed	% Leaf	% Stem	% Bark
Ash	4.8 ± 0.06	10.4 ±0.03	8.0 ±0.01	5.6 ±0.00
Crude fat	12.0 ± 0.05	11,0± 0.02	10.2 ± 0.04	11.8 ± 0.05
Crude fibre	15.6 ± 0.03	12.4 ± 0.01	12.0 ± 0.04	13.8 ± 0.02
Crude protein	14.0 ± 0.05	11.9 ± 0.00	12.3 ± 0.03	12.3 ± 0.01
carbohydrate	53.6 ± 0.04	54.3 ± 0.02	57.5 ± 0.00	56.5± 0.02
Energy value(kcal)	2.1 ± 0.04	3.2 ± 0.05	3.0 ±0.03	2.0 ± 0.01

Energy value (Kcal)[b]= (protein x 17 + fat x 37 + carbohydrate)

The leaves of investigated plant had highest amount of Ash. The fibre contents of the samples were comparably higher than other study (Amadi *et al*., 2017) while the seed contained highest fibre content. The fibre and ash contents under review were higher than what Amadi *et al*. (2017) reported for *A. indica* leaves extracts. This implies a possibly high mineral composition of the whole plant in this work. The results from the table 1 also revealed fat and protein compositions of seed samples to be high. These compositions were differed, as reported by Amadi *et al*. (2017) and Atangwho *et al*. (2009). Equally, carbohydrate was comparably higher than what was reported by Amadi *et al*. (2017)

The results for the phytochemical screening of *A.indica* plant extracts using petroleum ether, ethanol and aqeous were presented in tables 2-3. The results revealed the presence of saponins, tannins, flavonoids, alkaloids, anthracene glycosides and cyanogenic glycoside in all the *A. indica* solvent extracts. The phytochemicals were more concentrated and pronounced in leaves extracts than others particularly saponins which were mostly concentrated in extracts of both A. indica leaf and seed, this is in agreement with what reported by Biu *et al*. (2009). Other phytochemicals like tannins, flavonoids, alkaloids and glycocides were less concentrated in all the extracts of *A. indica* seed, stem and bark. A similar study reported absence of tannins and saponins in aqueous extract of *A. indica* (Ramadass and Subramanian, 2018) and glycosides (Amadi *et al.*, 2017) which are in contrary to our findings in this study.

Table 2 Phytochemical screening of *A. indica* leaf and *seed extracts*

PHYTOCHEMICAL	PET	EtE	WE	PET	EtE	WE
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Anthracene glycoside	+	+	+	+	+	+
Cynogenic glycoside	+	+	+	+	+	+

Table 3 Phytochemical screening of *A. indica* stem and bark extracts

Phytochemical	PET	EtE	WE	PET	EtE	WE
Saponins	+++	+++	+++	++	++	++
Tannins	++	++	++	+	+	+
Flavonoids	++	++	++	+	+	+
Alkaloids	+	+	+	+	+	+
Anthracene glycoside	++	++	++	+	+	+
Cynogenic glycoside	++	++	++	+	+	+

NB: +++ highly concentrate ++ moderate + slight. PET petroleum ether. EtE ethanol. WE aqueous

The presence of the phytochemicals in plants produces some physiological actions in man and animals, when ingested and it is responsible for their utilization as herbs in primary health care (Saradha and Subbarao, 2011). These phytochemicals also serve to protect the plant against infections by microorganisms, predations by insects and

herbivores, while their odour and flavour are responsible for their pigments (Mahmood *et al*., 2008) in most plants.

Alkaloids are known to confer several essential physiological effects on humans and animals. The presence of flavonoids indicates a possible antioxidant property of *A. indica*. The presence of saponins indicates possible usage of the plant extracts as a cleaning agent. The forkloric claims of this plant usage in medicine for the stimulation of the cardiac and uterine muscles in childbirth might be related to these alkaloid activities. *A. indica* leave is a very bitter tasting plant. This astringency could be as a result of tannin content. (Amadi *et al*., 2017)

Conclusion: The medicinal plants have been used for years in daily life to treat diseases all over the world. *A. indica* is a very useful traditional medicinal plant in the sub-continent. Each part of the tree has some medicinal properties. The present finding of proximate and phytochemical analyses of different extracts of *A. indica* will promote the utilization of the medicinal plants in various drug deliveries, therapeutic processes as well as in chemometrics. This study also has indicated the suitability of petroleum ether, ethanol and aqueous solvents for extraction of proximate and phytochemicals which saponins are more concentrated in leafy parts of the plant with indications of other

active constituents like, flavonoids, alkaloids and tannins.

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