Journal of Forestry Research and Management. Vol. 16(2).72-79; 2019, ISSN 0189-8418
www.jfrm.org.ng

Phytochemical and Elemental Analysis of the Leaves and Seeds of *Lawsonia inermis* linn Growing in Forestry Research Institute of Nigeria Herbal Garden

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ABSTRACT

Lawsonia inermis is known for its widespread uses ranging from traditional, cosmetics, pharmacological and phyto-pharmacologicals for the treatments of arrays of ailments and medical conditions. This study aims to unravel the elemental and secondary metabolite potential of *Lawsonia inermis*. Phytochemical analysis was carried out using standard procedures to determine the chemical composition in the leaves and seeds. Atomic Absorption Spectrophotometer was used to analyze the samples for its elemental composition. The phytochemicals detected in the leaves includes saponins, tannins, cardiac glycosides and alkaloidswhile flavonoids, saponins, tannins, cardiac glycosides were detected in the seeds. A total number of nine (9) elements were measured which had varying concentrations in both plant parts. For the leaves, the concentration were; Sodium (0.20mg/g), Potassium (2.00 mg/g), Magnesium(2.00mg/g), Calcium(1.67mg/g), Manganese(0.10mg/g), Iron(1.30mg/g), Copper(0.02mg/g), Zinc(0.08mg/g), Phosphorus(4.50mg/g)and for the seeds, Sodium (0.60mg/g), Potassium (4.00 mg/g), Magnesium(9.00mg/g), Calcium(15.00mg/g), Manganese(0.20mg/g), Iron(1.73mg/g), Copper(0.02mg/g), Zinc(0.09mg/g), Phosphorus(5.07mg/g). However, a high concentration of 15.00 mg/g, 9.00mg/g and 4.00mg/g respectively for Calcium, Magnesium and Potassium was obtained for *L.inermis* seeds. The results of this study revealed and corroborate the use of the leaves and seeds for phyto-pharmacology and as well justify its folkloric claims.

Keywords: *Lawsonia inermis*, phytochemicals, elemental, phyto-pharmacologicals, secondary metabolite

Introduction

Over the years, medicinal plants have been used across the world to cure various diseases as the basis for primary health care in the rural areas; this has led to an increasing interest in recent times in phytomedicines because they are relatively safe and more potent(Ekor, 2014). These medicinal plants are often prepared singly or in combination or even used as the principal source of raw materials (Lawal *et al.,* 2015). They are gaining popularity as alternative and complementary therapies around the world because human body is more accustomed to natural products. Therefore, scientific studies of medicinal plants are required to test their potentials and characterize their medicinal properties (Rayavarapu *et al.,* 2011). It is explained by World Health Organization (WHO) that medicinal plants would be the greatest source and origin of contents of a large variety of drugs. Therefore, medicinal plants should be investigated to understand their activities, safety and efficacy properties for people using them (Rayavarapu *et al.,* 2011). This has led to the research on *Lawsonia inermis* which is one of the most popular plant known with these features, and it is now the subject of several scientific studies (Barbieri *et al.,* 2017).

Lawsonia inermis is a plant that is majorly distributed across the Sahel and Central Africa. It also exists in the Middle East and

has a wide distribution across the Northern and Southern parts of Nigeria (Orwa *et al.*, 2009). This plant is cultivated in Africa and Asia for both medicinal and industrial (dyeing) purposes and cultivated commercially throughout Pakistan, India, Iran, Libya and Sudan for its valuable leaves (Saadabi, 2007; Zumrutdal and Ozaslan, 2012).The plant is commonly known as "Henna" and belongs to the family "Lythraceae" and the species is sometimes classified as *Lawsonia alba* Lam. Or *Lawsoniaruba* L. Henna plant is deciduous, has a perennial shrub which is reaching a height of up to 5metres. The plant leaves are small, lanceolate, dark green, opposite and have short petioles. *Lawsoniainermis* L. is recognized in traditional system of medicine and consists of several phytochemicals like flavonoids, steroids, coumarins, xanthones and triterpenoids. Phytochemical screening of *L.inermis* has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, glycosides, saponins (Ibrahim *et al.,* 2008). The plant contains various compounds like; gallic acid, coumarins, naphthalene derivates, flavonoids, sterols, triterpenoids, tannins, saponins, glycosides, and xanthones (Muhammad and Muhammad, 2005;Chaudhary*et al.,* 2010).The leaves of the plant contain a red orange color component, lawsone (2-hydroxy-1, 4-Napthoquinone). Lawsone (2-hydroxy-1, 4-Napthoquinone) is easily bonding with protein, and thus it has been used to dye skin, hair and fingernails (Siddiqui *et al.*, 2003, Rahiman and Taha, 2011).A large number of studies reported that henna is used for headache, lumbago, bronchitis, syphilitis, scabies, sores, amenorrhea, diarrhea, bleeding disorder, diuretic, skin diseases, anti- amoebiasis, antibacterial, antifungal, sedative, astringent, anti-hemorrhagic, and hypotensive effect (Borade *et al.*, 2011). In traditional medicine, henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, hemorrhoids and even in jaundice, leprosy, pain, spleen enlargement, dysentery and skin problems (Bhuvaneshwari *et al.*, 2002; Cuong *et al.*, 2009;Rahmoun*et al.,* 2010). The leaf powder of *L.inermis* is used for staining hair, nails and beard (Chengaiah*et al.*, 2010).The powdered roasted seed is mixed with gingerly oil to make a paste which is used for the treatment of ringworm. An ethnobotanical survey in the South Western part of Nigeria reported that majority of the people make use of *L.inermis* leaves as an anti-malaria with majority of people having preference for the aqueous extract, also it was reported that methanolic root extract is used in Nigeria for cosmetic purposes (Idowu *et al.,* 2010).

The seeds have been reported to have antioxidant activity and to being a strong antioxidant substance and was confirmed to mitigate or prevent generation of free radicals(Phillip *et al.,* 2011). It is considered a safe herbal medicine with only few and insignificant adverse/ side effects (Wagini *et al.,* 2014).

Inorganic elemental levels present in medicinal plants are of great importance due to their pharmacological actions (Lokhande *et al.,* 2010). Many of these plants are believed to contain elements of vital importance in metabolism and that are needed for growth and development (Obiajunwa *et al.,* 2002).

Active constituent of medicinal plants are metabolic products of plant cells and a number of trace elements play an important role in the metabolism (Rajukar and Damame, 1997). Thus, the screening of the actual bioactive elements of plant origin and assessment of elemental composition of widely used medicinal plants is highly essential.

In the present study, an effort was made to determine the plant secondary metabolites present as well as the presence of macrominerals (Ca, Na, K, Mg, P), microminerals (Fe, Cu, Zn, Mn), in *L. inermis* leaves and seeds by using Atomic Absorption Spectrophotometer (AAS), This is an area of investigation that there are dearth reports on the plant.

Materials and methods

Collection, Identification and Preparation of *Lawsonia inermis* Leaves

Fresh leaves and seeds of *Lawsonia inermis* were collected from the Forestry Research Institute of Nigeria Herbal garden located in the South-Western part of Nigeria. The samples were identified by a plant taxonomist and the voucher specimens were deposited in the Forestry Research Institute of Nigeria Herbarium. The leaves were dried at 40°C in a heat controlled oven until they attained the required dryness. The samples were then blended to powder, using a manual grinding machine.

Phytochemical Screening

Test for Saponins

0.5g of the powdered sample was added to 5ml of distilled water in a test tube and the solution was well shaken and observed for a stable persistent froth. To the froth, 3 drops of olive oil was added and shaken vigorously after which it was observed for the formation of an emulsion in accordance with (Otang *et al.*, 2012; Lawal *et al.*, 2015).

Test for Tannins

0.5g of the powdered sample was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution was observed for brownish green or a blue-black colourationin accordance with (Otang*et al.*, 2012; Lawal *et al.*, 2015).

Test for Anthraquinones

0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipetted into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for color changes in accordance with (Otang*et al.*, 2012; Lawal *et al.*, 2015).

Test for Cardiac Glycosides (Keller-Killiani Test)

0.5g of extract was dissolved in 5ml distilled and filtered. To the filtrate was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxy sugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer in accordance with (Otang *et al.*, 2012; Lawal *et al.*, 2015).

Test for Flavonoids

0.5g of the powdered sample was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was mixed with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids in accordance with (Otang *et al.*, 2012; Lawal *et al.*, 2015).

Test for Alkaloids

0.5g of the powdered sample, 10ml of acid alcohol was added, boiled and then filtered. To 5ml of the filtrate, 2ml of Dilute Ammonia, then 5ml of chloroform was added and gently shaken to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. To one portion, Mayer`s reagent was added and

Journal of Forestry Research and Management. Vol. 16(2).72-79; 2019, ISSN 0189-8418
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Dragendroff`s reagent was added to the other. The formation of a cream precipitate (with Mayer`s reagent) or reddish brown precipitate (Dragendroff reagent) was regarded as positive for the presence of alkaloidsalkaloids. This was carried out in accordance with (Otang *et al.*, 2012;Lawal *et al.*, 2015).

Methods for elemental analysis

Major and trace elemental contents were estimated using model 210VGD Buck Scientific Atomic Absorption Spectrophotometer at the Bioscience Department, Forestry Research Institute (FRIN), Ibadan, Nigeria. The instructions for setting up the equipment, calibration and sensitivity for specific elements as laid down in the operational manual were strictly followed (Zafar *et al.*, 2010)

Results

The Phytochemical characteristics of the leaves and seeds of *L. inermis* investigated are summarized in Table 1.The results reveal the presence of medicinally active constituents in the plant parts studied. From Table 1, saponins, tannins, flavonoids, cardiac glycosides were present in the two plant parts, alkaloids were absent in the seeds while anthraquinones were absent in both plant parts.

Table 1: Phytochemical Constituents of the Leaves and Seeds of *L. inermis*

Chemical constituents	Leaves	Seeds
Tannins	+	+
Saponins	+	+
Anthraquinones	-	-
Alkaloids	+	-
Flavonoids	+	+
Cardiac Glycosides	+	+

Table 2: Elemental Analysis of the Leaves and Seeds of *Lawsonia inermis*

S/N	Element	Leaves(mg/g)	Seeds(mg/g)
1.	Sodium	0.20 ± 2.78^a	0.67 ± 0.21^a
2.	Potassium	2.00 ± 0.22^a	4.00 ± 1.63^a
3.	Magnesium	2.00 ± 0.14^a	9.00 ± 1.41^a
4.	Calcium	1.67 ± 0.17^a	15.00 ± 2.94^a
5.	Manganese	0.10 ± 0.01^a	0.20 ± 0.08^a
6.	Iron	1.30 ± 0.22^a	1.73 ± 0.21^a
7.	Copper	0.02 ± 0.01^a	0.02 ± 0.01^a
8.	Zinc	0.08 ± 0.02^a	0.09 ± 0.02^a
9.	Phosphorus	4.50 ± 0.16^a	5.07 ± 1.23^a

Values expressed as Mean ± Standard Deviation

Discussion

The results of the phytochemical screening of the leaves and seeds of *L. inermis* showed the presence of various secondary metabolites such as flavonoids, alkaloids, saponins in the leaves which is in accordance with the report given by (Yusuf, 2016). Also tannins was present which also agrees with the results reported by (Raja *et al.*, 2013) while cardiac glycosides and anthraquinones were absent in both plant parts which was in accordance with the results of (Yusuf, 2016) and alkaloids absent in the seeds (Table 1). These findings suggest that this plant is a potential source of alternative medicine that could greatly be useful as therapeutics for different diseases.

The data obtained as cited in Table 2 shows that the concentration of the elements; K, Ca,Mg, Fe and P were highest in the leaves and seeds with the seeds being rich in K, Ca, Mg, P with values (4.00, 15.00, 9.00, 5.07, 16.80)mg/g respectively. The values obtained for K, Mg in *L. inermis* leaves are within range to the values obtained for *Fagonia indica* with values for K (2293ppm), Mg (1819.5ppm) and Na (155.3ppm) (Zafar *et al.*, 2010). Also, in *L. inermis* seeds, concentration of Ca was the highest with value 15.00mg/g while Mg and K concentration in the seeds was also relatively high with values 9mg/g and 4mg/g respectively This denotes that the seeds has the potential to help promote good bone health which is characteristic of Calcium which when consumed adequately helps to promote this function and also helps the prevention of osteoporosis, major food sources of Ca include dairy product, leafy vegetables, and beans (Clarkson and Haymes, 1995).The seeds can also reduce blood pressure and regulate fluid balance because of the presence of Potassium which can be found in foods such as banana, avocado, citrus fruits, leafy, green vegetables, milk, potatoes (Manore, 2001). The existence of Magnesium in the seeds as observed shows that it has potential to help influence bone metabolism and Mg is predominantly high in foods like nuts, seafood, green leafy vegetables, other fruits and vegetables, black beans and whole-grain products (Sojka and Weaver, 1995). Also Sodium present in the seeds shows that it can be used for muscle contractions, nerve transmissions, maintaining pH balance and hydration, food sources includes fish, beans, meat, whole grain, sea food (Manore, 2001).However, the concentrations of Mn, Cu and Zn were relatively low in both plant parts studied (Table 2) but not as low as values obtained for same elements in *Fagonia indica* (Mn- 32.2ppm, Cu- 1.8ppm, Zn- 0.42ppm) as reported by (Zafar *et al.*, 2010). Manganese (Mn) functions in the body in energy metabolism, bone formation and fat synthesis, major food sources of manganese are wholegrain products, dried peas and beans, leafy vegetables (Odewale and Lawal., 2017). Copper (Cu)is an essential mineral whose function is closely associated with the function of iron in oxygen metabolism. Food sources of Cu includes seafood, meats, nuts, beans, and whole-grain products. energy and oxygen metabolism (Lukasi, 1995) and Zinc (Zn) is found virtually in all tissues in the body and is required for the activity of more than 300 enzymes and a wide variety of other body functions such as bone formation and wound healing (Michelleti, 2001). This implies that *L. inermis* leaves and seeds have a better potential in making up for the deficiencies of some of these elements in human body when ingested. The levels of these elements in both plant parts are not significantly different at $p<0.05$. These results connotes that the plant part is highly of great potential medicinally.

Conclusion

The data obtained in this study revealed that the leaves and seeds have high medicinal potential when explored based on the presence of the wide array of plant secondary metabolites as well as the minerals found in them. The role of these macro and micro minerals in the body has been well established, thus their existence in the studied plant parts helps us to better understand and appreciate its pharmacological potential and uses. Also, the data generated from this study will contribute to pharmacopeia information of *L. inermis* and can serve as reference and standards which can be a useful lead in the synthesis of new herbal drugs used in the management and treatment of different kinds of diseases..

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