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ORIGINAL RESEARCH ARTICLE

Phytochemical screening and antioxidant activities of the bark of *Lawsonia inermis* (Henna) grown in Dekina, Kogi, Nigeria

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ABSTRACT

Severe health conditions can result from lack of knowledge on the bioactive constituents of medicinal plants, as it the main stay of treatment and prevention of diseases in our locality. Hence, the need to examine the therapeutic active ingredients responsible for plants medicinal ability for better health management. This study analyses the phytochemical constitutes and anti – oxidant activity of the bark of *lawsonia inermis*. *Lawsonia inermis* bark was collected, cut into pieces and dried at room temperature. The dried sample was pulverized into fine particle. 200g of the pulverized sample was weighed into a 750mL container and was added 300mL absolute ethanol, then allowed to stand for extraction. The solute obtained by evaporating the ethanol was screened for the presence of phyto – components and the result shows alkaloids, glycosides, saponins, phenols, flavonoids, proteins and amino acid as bioactive substances. Also, *lawsonia inermis* bark displayed an efficient free radical scavenging potential as the anti – oxidant ability showed the percentage $KMnO_4$ radical scavenging activities of 41.4% which shows close competence with the standard ascorbic acid; 47.9% at EC_{50}; This indicate *lawsonia inermis* bark to exhibit a good potential as antioxidant and could scavenge free radical from the body. The presence of the bioactive substances signifies its usefulness in therapeutic health as agent that could inhibit diseases causing several ailments and as well be a good source for health management control.

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INTRODUCTION

Management of plants in disease treatment and prevention have been in use over a long period of time, i.e. since ages (Mohan, *et al.*, 2021), with communities been ignorant of the active ingredient responsible for the potency. Plants are used in the treatment of diseases like hypertension, pile, typhoid fever, asthma, rheumatism etc. (Mohan, *et al.*, 2021; Farzama, *et al.*, 2021 and Nguyen, *et al.*, 2021). Medicinal plants are known to possess the richest bio resources such as anti-neoplastic, antimicrobial, antioxidant, anti-inflammatory etc. (Junaid, *et al.*, 2020; Farzama, *et al.*, 2021; Mohan, *et al.*, 2021; Letiele, *et al.*, 2021; Isah, *et al.*, 2020; Nigam & Arnold, 2020). Their medicinal potential is a result of natural secondary metabolites which include flavonoids, saponins, tannins, alkaloids, phenols etc. that fight against bacterial infections and related diseases (Farzama, *et al.*, 2021). These secondary metabolites are known as phytochemicals and they work in conjunction with nutrients and dietary fiber to protect the body from diseases and infections; they also contribute to regeneration of cells in the body (Isah, *et al.* 2020). Plants inherent ability to biosynthesize a diverse range of non-enzymatic antioxidants capable of mitigating oxidative damage is attributed to low progression of several diseases caused by free radicals (Deepak, *et al.*, 2015), they also have evolved a variety of active substances that scavenge free radicals and thus protect cells, delay aging and protect diseases associated with aging (Hassan, *et al.* 2017), these substances can significantly reduce or inhibit the rate of oxidation and participate in physiological processes and also terminate chain reaction caused by free radicals that are linked to diseases like cancer, arthritis, liver damage, and diabetes (Olamide, *et al.*, 2017; Shohber, *et al.*, 2015). The role of plants as antioxidant have shown its ability to remove free radicals from nerves and thus effectively protect against most nervous system diseases. Bioactive substances such as polyphenols, vitamins, alkaloids, polysaccharides, and active peptides in plants aids in the preservation of neuron structure and the maintenance of a healthy state

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have been the major sources of antioxidants used in reducing oxidative stress (Muhamat, et al. 2021). These potentials are also attributed to Lawsonia inermis.

Lawsonia inermis is commonly known as hanna, it belongs to the kingdom plantae, phylum; spermatophyte, sub phylum; angiosperm, class; dicotyledon, order; myrtales and family; lythraceae (Rojas – Sandovaj (2017). It is a plant that have been part of human existence for curbing many diseases. Researches conducted by scholars indicted the significant medicinal importance of *Lawsonia inermis* in the treatment and management of diseases such as leprosy, fever, leucorrhoea, rheumatoid, arthritis, ulcer, cardiac disease and headache (Arirudran, *et al*., 2019; Chetty, 2008; Reddy, 1988). The medicinal value of *Lawsonia inermis* also found significance in the treatment of wounds, blood sugar, microbial infections, inflammations and proliferation of cancer cells (Debrayiya, *et al*., 2020). The healing properties like analgesic, hypoglycemic, hypotoprotive, immuno stimulant, anti-inflammatory, antimicrobial, antibacterial, antifungal, antivirus anticancer, anti-parasitic, anti- dermatophytic properties etc. were attributed to the presence of phytochemicals components like carbohydrate, proteins, flavonoids, tannins and phenolic compounds (Gagandeep, *et al*., 2010). Phytochemicals substances; flavonoids, steroids, tannins, protein, cardiacglycosides, alkaloids and carbohydrate were reported on the flowers of *lawsonia inermis* to be responsible for it therapeutic potentials (Syede, 2018). The radical scavenging ability denotes the inhibition potential of the plant bark with strong antioxidant capabilities and medicinal role in combating diseases causing different ailment (Dharmesh, *et al*., 2020). Traditional medicine is in constant use as a natural remedy in our locality for the treatment of several ailments, with the community having little or no knowledge of the active ingredients responsible for its medicinal potency, hence, the need to elucidate the phytochemical components of this plant part. Despite the important role of *Lawsonia inermis* as a source of natural antioxidant in disease treatment and health management in our society, hazard attributed to drug toxicity that could lead to severe health conditions and possible death could arise as a result of overdose and basically lack of understanding of the phyto – constituents. This study is aimed at investigating the phytochemical substances and antioxidant activity of the bark of *Lawsonia inermis*.

MATERIALS AND METHODS

The materials used include pulverized *lawsonia inermis* bark which was extracted using absolute ethanol (99%), sodium hydroxide, ammonia solution, hydrochloric acid, ferric chloride solution, concentrated sulphuric acid, nitric acid, Hager reagent, Gelatin solution, sodium nitroprusside, pyridine, Benedict solution were standard analytical reagents used for the phytochemicals analysis. $KMnO_4$, Ascorbic acid, Hydrogen peroxide and double beam UV / Vis spectrophotometer 2700

Collection and sample Treatment

Bark of *Lawsonia inermis* was collected in Dekina, Kogi State and was identified and authenticated by a botanist, Professor Simon Sugei Usman and the sample deposited at our institution department of Biology. It was cut into pieces and air dried at room temperature. The plant part was then finely ground. 200g of the pulverized sample was weighed into a 750mL plastic container, followed by 300mL of absolute ethanol and corking. For proper extraction, the mixture was left for a week. The extract was filtered, and the dried solute was sacrificially obtained (Leonard, *et al*., 2022).

Phytochemical screening

Phytochemical screening method employed are as described by Prashant *et al.* (2011); Junaid & Pati (2020).

Test for alkaloids

Hager's test: the extract (2g) was weighed and dissolved in 5mL diluted HCl and filtered. 1mL of Hager's reagent was mixed with 2mL of the filtrate. The presence of alkaloids will be confirmed by a creamy white precipitate.

Test for saponins

Foam test: sample of the extract, (1g) was dissolved in distilled water and vigorously shaken before being allowed to stand for 10 minutes. Saponins will be detected by a stable persistent foam.

Test for phytosterols

Salkowski's test: the extract was weighed (2g) and treated with chloroform and then filtered; the filtrate was then treated with a few drops of concentrated H_2SO_4; the mixture was shaken and allowed to stand until a golden yellow color appeared.

Test for flavonoids

Alkaline reagent test: few drops of sodium hydroxide solution was added to 2g of the extract, formation of an intense yellow color that will turn colorless upon addition of dilute HCl acid solution will confirm the presence of flavonoids.

Test for carbohydrate

Benedicts test: the plant extract, (2g) was weighed and 5mL distilled water was added and dissolved, then filtered. A 0.2 mL sample of the filtrate was treated with Benedict reagent and gently heated. The presence of carbohydrate will be confirmed by the formation of orange-red precipitate.

Test for tannins

Gelatin test: the extract (0.2g) was weighed and dissolved in 5mL of distilled water, followed by 1% gelatin solution and 10% sodium chloride solution. The presence of tannins will be indicated by a white precipitate.

Legal's test: the plant extract, (0.5g) was weighed and dissolved in pyridine, sodium nitroprusside, and 10%

sodium hydroxide. When glycosides are present, the solution will turn pink.

Test for glycosides

Legal's test: the plant extract, (0.5g) was weighed and dissolved in pyridine, sodium nitroprusside, and 10% sodium hydroxide. When glycosides are present, the solution will turn pink.

Test for phenols

Ferric chloride test: the plant extract, (0.2g) was treated with 3-4 drops of ferric chloride solution. The presence of phenols will be indicated by the formation of a bluish-black color.

Test for protein and amino acids:

Xanthoproteic test: the plant extract, (1g) was treated with few drops of concentrated nitric acid. Formation of yellow color will indicate the presence of protein.

Antioxidant Activities

Radical scavenging method using acidified potassium permanganate ($KMnO_4$) was used. Ascorbic acid at concentration of 2.0mg/mL – 0.25mg/mL (I e 2.0, 1.5, 1.0, 0.5 & 0.25mL) was used as standard antioxidant. 0.5mL of acidified potassium permanganate solution and 0.5mL of hydrogen peroxide was added as pro oxidant. Absorbance of both the $KMnO_4$/ascorbic acid and $KMnO_4$/sample extract were taken spectrophotometrically at a wavelength of 520nM after 30 minutes' incubation period. The graph of absorbance against concentration was then plotted. The % $KMnO_4$ scavenging effect was determined using

% $KMnO_4$ scavenging effect = Ac – At/Ac * 100/1

Where:

Ac = Absorbance of control

At = Absorbance of the tested sample (Isaac *et al.*, 2011)

Preparation of standard solutions

Ascorbic acid: (0.011 mol/ 100mL),

0.2g of ascorbic acid was weighed and dissolved in 100mL of distilled water.

Potassium permanganate: (0.0006 mol/100mL)

0.01g of potassium permanganate was weighed and dissolved in 100mL of distilled water to make the stock, from the stock 25mL of $KMnO_4$ solution was acidified by adding 10mL of 2M H_2SO_4 solution.

RESULTS

Phytochemical screening of *lawsonia inermis* bark

The phytochemical substances identified are alkaloids, glycosides, saponins, phenols, flavonoids, protein and amino acid, while Carbohydrates, phytosterols and tannins were absent as shown in Table 1.

Antioxidant activity using %$KMnO_4$ scavenging effect

The % $KMnO_4$ scavenging effect for the sample is 41.4% and that of the standard is 47.9% at EC_{50}. Fig.1 below shows the graphical competence of the bark of *lawsonia inermis* and indicate its antioxidant properties.

Table 1: Phytochemical screening Result

Parameters	Test performed	Observation	Results
Alkaloids	Hager test	Creamy white precipitate formed	+
Carbohydrates	Benedicts test	Orange – red precipitate formed	-
Glycosides	Legal test	Pink solution formed	+
Saponins	Foam test	Persistent foam formed	+
Phytosterols	Salkowskis test	Golden yellow solution formed	-
Phenols	Ferric chloride test	Bluish – black colour formed	+
Tannins	Gelatin test	White precipitate formed	-
Flavonoids	Alkaline test	Intense yellow color turns colorless in dil HCl	+
Protein and amino acid	Xanthoproteic test	Yellow color formed	+

Key: (+) = positive; (-) = absent

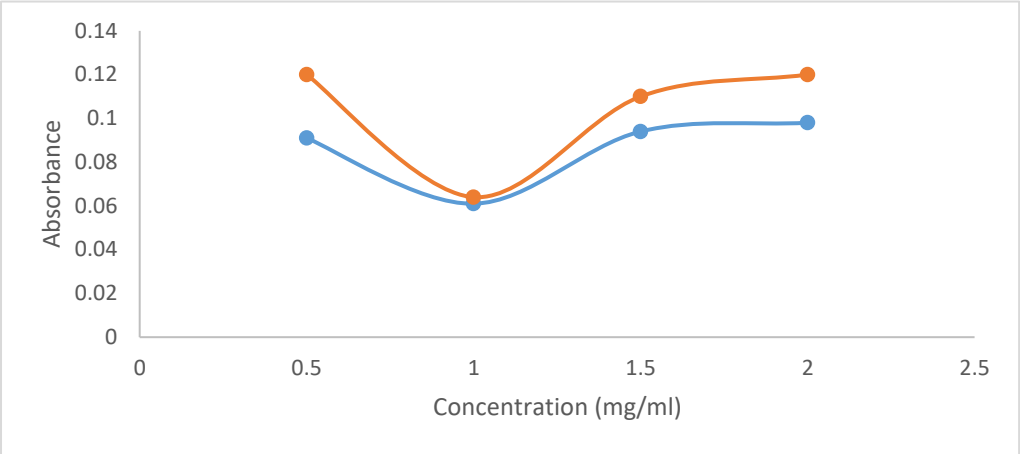

Figure 1: Result of the antioxidants activities of *Lawsonia Inermis* bark

DISCUSSION

Phytochemical screening result of this study show the therapeutic potential of the bark of *Lawsonia inermis* plant as a result of the presence of bioactive substances in it. Previous work conducted on this plant also present bioactive ingredients, although with some variation from the current study. A work conducted at Lahore, Pakistan by Iram *et al.* (2013) indicted the presences of cardiogycosides, terpernoids, carbonhyrate, phenols, quinones and tannins with the absent of protein on the methanol, acetone and aqueous extract of the leaves. Nirmala *et al.* (2016) reported the methanol extract to contain carbohydrate, protein, flavonoids, tannins, phenolic compounds, alkaloids, terpernoids, glycoside, saponins, quinones, protein and amino acid in a study they conducted at Telegona, India. Gabriel *et al.* (2017) reported a quantitative presence of total alkaloids, total flavonoids, total phenol, total saponin and total tannin with glycosides and protein been absent in a research they carried out on the ethanolic extract of the stem bark of *Lawsonia inermis* in Ogun, western Nigeria. In another work by Dharmesh *et al.* (2020) in india, flavonoids, polyphenols, tannins, and terpenoids were reported to be present in the methanol extract of the bark of *lawsonia inermis* plant with alkaloids, saponins, proteins and glycoside to be absent.

The variations in the presence of the bioactive constituents as seen in the results could be as a factor of the extracting solvents used, as different extracting solvent possesses different polarities. Also, phyto - substituents are related to their geographical origins; the geographical location or origin of plants as reported by Mohammed and Aisha (2022) could contribute to the variation in the phyto – component present in the plant. The antioxidant predicts the plant bark to possess a great potential to scavenge free radical, this finding agrees with Debapriya *et al.* (2020) in a work they uphold the radical scavenging potentials of *Lawsonia inermis* in treating human disease like cancer. In a similar vein, Dharmesh *et al.*, (2020) affirm the anti – oxidative activity of the plant in their study where *Lawsonia inermis* bark indicted inhibitory capacity in DPPH, Superoxide and Ferric chloride methods of free radical scavenging activity.

CONCLUSION

Plants have been in use as medicine over decades in the treatment of different kind of illnesses; despite the advancement in modern medicine, traditional medicine which greatly utilizes plants is still gaining significance; this is so because of its preventive and curative potential which is as a result of the presence of bioactive substances. This study has shown the presence of alkaloids, glycosides, saponins, phenols, flavonoids, protein and amino acid as bioactive substances. They phyto – chemical constituents and the efficient free radical scavenging ability displayed by the bark of *Lawsonia inermis* indicates that it could inhibit oxidation process and possibly prevent oxidative damage on cells and tissues. This study affirms the presence of therapeutic substances responsible for its efficacy when use as traditional medicine. The plant possesses medicinal properties and if properly exploited, it can potentially be used to treat ailment caused by free radicals and also it could significantly serve as substitute agent in therapeutics. However, standardization and isolation into pure form and the investigation of the toxicity level is recommended for better and efficient health management control.

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