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Phytochemical Analysis of Green Plant Extracts

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ABSTRACT: *Green Plant Extracts are very vital in combating bacteria and fungi. They also have inhibitory effect on Mild Steel (MS) because of their microbial properties. Plant extracts are used both in developed and developing countries as home remedies. In this investigation, Ocimum Gratissimum, Manihot Esculenta and Azadirachta Indica are utilized to determine their phytochemical propertiesusing qualitative and quantitative methodsSome prominent bioactive constituents of the plants like alkaloids, tannins, flavonoids, saponins and phenolic compounds were revealed. Tannin was more and present in all the plant leaf extracts.*
KEY WORDS: Phytochemical, bioactive constituent, mild steel, natural green plants.

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I. INTRODUCTION

Plants are major sources of medicine from the inception of the creation of the universe. The Creator of the universe commanded that plants (herbs) should be used for healing. According to the Holy Bible, the leaves of the tree were for the healing of the nation and the fruits will be for food [1]. Since then, plants have been effective medicinal products for healing. According to Priya *et al*., [2], medicinal plants represent a rich source of antimicrobial agent and are used as a source of many potential and powerful drugs in several countries [3]. They are identified and used throughout human history and have the capacity to synthesize wide variety of chemical compounds that are used to form important biological functions. Also, they are effective in the defend against attacks from predators such as insects, fungi and herbivorous mammals [4]. The viability of plants is inexhaustible to mankind in all facets of engagement. Chopra *et al*., [5] stated that plants produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. Traditionally, medicine derived from plants play principal role in the health care system of numerous countries like India, China, Nigeria, etc. [2]. The medicinal plants have some chemical active substances that produce a definite physiological action on the human body. The most prominent of these bioactive constituents of the plants are alkaloids, tannins, flavonoids, saponins and phenolic compounds [4]. However, these bioactive chemical constituents also play significant roles in inhibiting corrosion on Mild Steel (MS). Moreover, they possess the properties of non-toxicity, environmentally begnin and less costly. Economic and safe environment are the main merits for using them [6,7,8]. These contributed much relevance in their usage as corrosion inhibitors as compared to inorganic substances such as phosphates, chromates, dichromate and arsenates on Mild Steel [9].

Ocimum Gratissimum called Scent Leaf or Clove basil is found in tropical countries. It has many medicinal uses and values. The values depend on certain active chemical or bioactive substances such as tannins, oligosaccharides, phenols, flavonoids and alkaloids [10]. Medicinally, it can be used in the treatment of cough and catarrh when inhale, stomach pain, diarrhea, cholera, chronic dysentery and vomiting. Also, it can be infused for treating urinary infections and gonorrhea. The oil in it can be used for food preservation because of its antimicrobial and antibacterial properties. The oil in the leaves contains antifungal, antibacterial and antiseptic properties [11,12].

Manihot Esculenta is popularly known as Cassava, manioc, yucca, mandioca and Brazilian arrow root [12,13] a woody shrub nature to South America of the spurgy family, Euphorbiaceae. Cassava is mostly cultivated as an annual crop in tropical and subtropical regions. It is mostly planted because of its edible starchy tuberous root which major source is carbohydrate [12,14].

Azadirachta Indica otherwise called Neem, Nimtree or Indian lilac [12,15] is a tree belonging to the mahogany family, Meliaceae. Azadirachta is found in Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka and Maldives, and also grown in Iran Island. Like Manihot Esculenta, it is grown in tropical and subtropical regions. Its oil is derived from the fruits and seeds. Much consumption of the oil quantity causes toxic encephalopathy and ophtalmopathy [12,16]. It has as a bitter taste which the leaf or bark as an effective pitta pacifier. Its bark contains 14% tannin. The oil in it averts termite attack as ecofriendly and

economical agent [12,17]. It is used for the treatment of malaria. It can also be used as a nitrification inhibitor when the extracts are blended with fertilizer (urea) [12,18].

II. MATERIALS AND METHOD

The phytochemical analysis was carried out in the Central Research Laboratory, Niger Delta University, Wilberforce Island, Bayelsa State in Nigeria to determine the presence and percentages of radicals, such as: Alkaloid, Tannin, Saponin, Flavonoid and Phenol in the green leaf extracts of Scent Leaf, Cassava Leaf and Neem Leaf. They were analysed by quantitative and qualitative methods. The filtrates were used for the phytochemical screening applying the usual procedure described by Harbone (1973), Boham and Kocipal-Abyazan (1974) and Edeoga *et a*l., (2005). Tannin by Van-Burden and Robinson (1981) method, saponin by Obadoni and Ochuko (2001) method, flavonoid by Boham and Kocipal-Abyazan (1974) method [18].

Qualitative phytochemical analysis determines the presence of the radicals in the green leaf extracts. Table 1 shows the results of the qualitative analysis on the samples. While quantitative phytochemical analysis determines the percentage of the radicals in the green leaf inhibitors. Table 2 shows the percentage of the various radicals in the leaf extracts. In the qualitative analysis, the various dried grinded green leaves were added and dissolved in various reagents according to the set standards. The test tubes were immersed in water flask and placed on hot plate to heat the reagent and the green leaf inhibitor. The test tubes were retrieved and filtered in Whatman filter paper. The residual was examined to ascertain the colour of the type of radical in the green leaves. However, comprehensive methods are given below.

2.1 Qualitative Phytochemical Analysis

Qualitative analysis was carried out using Weighing balance (Electronic), Heating Apparatus (Hot Plate), Volumetric Flask, Beaker, Boiling Tubes, Conical Flask, etc.

2.1.1 Test for Alkaloids [12]

0.2g of evaporated extract was boiled with 5ml of 2% HCl on a steam bath for 5 minutes. The mixture was filtered after cooling. The filtrate was divided into three test tubes A, B and C. Test tube A, 1ml portion of the filtrate was treated with 2 drops of Mayer's reagent, a creamy white precipitate was observed. This was confirmed with 1ml of the filtrate treated with 2 drops of Dragendorff reagent which gave a red ppt to indicate the presence of Alkaloids.

2.1.2 Test for Tannins [12]

0.2g of powdered samples, 5ml of 45% ethanol was added and boiled in a water bath for 5 minutes. The mixture was cooled and filtered. To 1ml of filtrate, 3 drops of lead acetate solution was added. The formation of gelatinous ppt indicates the presence of Tannins. Also, as a conformation test, 1ml filtrate was treated with 0.5ml of bromine water and the formation of pale brown ppt indicates the presence of Tannins.

2.1.3 Test for Saponins [12]

0.1g of extract was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while hot. 1ml of the filtrate was treated with 2 drops of olive oil and the mixture was shaken. The formation of emulsion was observed. Another 1ml was shaken with 4ml of water and the formation of a stable frothing on standing indicates the presence of Saponins.

2.1.4 Test for Flavonoids [12]

0.5g of the extract was introduced into a test tube. 10ml of ethyl acetate solution was also added and the mixture was heated in a water bath for 1 minute. The mixture was filtered and 4ml of the filtrate was treated with 1ml of 1% Aluminum Chloride ($AlCl_3$) solution and left to stand for 10 minutes. The formation of a yellow coloration in the presence of 1ml of diluted ammonium hydroxide indicates the presence of Flavonoids.

2.1.5 Test for Phenols [12]

0.10g of extract was treated with 3 drops of ferric chloride solution. The formation of a blue-black colour indicates the presence of Phenols. Also, 0.10g of extract was treated with 3 drops of lead acetate solution. The formation of a yellow-coloured solution ppt indicates the presence of Phenols.

2.2 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was conducted by using Petroleum (Ether), Sulphuric Acid (Conc.), Sodium hydroxide, Anhydrous Sodium Sulphate, Anhydrous Copper Sulphate, Mayer's Reagent, Dragendorff Reagent, Ethyl Acetate, Aluminum Chloride, Ammonia Solution, Olive Oil, Ethanol (45%), Ferrous Sulphate, Ferrous Chloride, Lead Acetate, Bromine water, Fehling Solution A & B, Chloroform, etc. [19].

2.2.1 Determination of Alkaloids [12,20]

5g of powdered plant parts were placed in 250ml beaker and 200ml of 10% acetic acid was added and covered with aluminum foil and allowed to stay for 4 hours. This was filtered and the extract was concentrated to about ¼ of its original volume on a water bath. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation, ppt was completed. The solution was allowed to settle. The precipitate was collected over a Whatman No1 filter paper and further washed with dilute ammonium hydroxide. The residue was dried in the ovum at 65^{o}C until completely dried. It was then weighed as the alkaloids obtained.

$$\% \, Alkaloid \; = \frac{b}{a} \times \frac{100}{1} \qquad 1$$

Where, a = weight of sample
b = weight of dried ppt

2.2.2 Determination of Flavonoids [12,21]

10g of powdered plant samples were repeatedly extracted with 100ml aliquots of 80% aqueous of methanol at room temperature. The extract was filtered through Whitman No 42-filter paper. The filtrate obtained was transferred into porcelain crucible and evaporated to dryness over a water bath, weighed up to constant weight. This gives the percentage content of flavonoids.

$$\% \, Flavonoid \; = \frac{b}{c} \times \frac{100}{1} \qquad 2$$

Where, b = wt of ppt
c = Sample wt.

2.2.3 Determination of Saponins [12,18,19]

10g of powdered plant part were weighed into 100ml conical flask and was added with 50ml of 20% aqueous ethanol. The sample was placed on a water bath at 550^{o}C with continuous stirring for 4 hours. The mixture was then filtered. The residue was also re-extracted with another aliquot of aqueous ethanol. Both extracts were combined and concentrated down to 40ml over a water bath kept at 90^{o}C. The resulting concentration was transferred into 250ml separating funnel and 10ml aliquots of diethyl ether was added and shaken vigorously. Two layers were formed in the funnel, which the aqueous layer was recovered and the ether layer was discarded. The aqueous layer then re-extracted with 30ml of n-butanol. A combined n-butanol was washed with aqueous NaCl solution twice. The remaining solution was placed on a water bath and heated to dryness. After evaporation, the residue obtained was dried in an oven to a constant weight and the saponins percentage was calculated.

2.2.4 Determination of Phenols[12]

5g of the powdered samples were defatted by staking the leaves in a petroleum spirit for 1 hour. By way of soaking and filtering, the leaves were defatted. These were boiled in 50ml of ether for 15 minutes to extract the phenolic fraction. 5ml of the extract was transferred into a 50ml volumetric flask; 10ml of diluted water was added, followed by 2ml of ammonium hydroxide. A further 5ml of fresh amyl alcohol was added.

III. RESULTS

3.1 Qualitative phytochemical analysis

The preliminary study of the qualitative analysis of the green leaf extracts as shows in Table 1 revealed that in Scent Leaf, alkaloid is more, tannin is more, saponin is none, flavonoid is less and phenol is less. In Cassava Leaf, alkaloid is less, tannin is abundant, saponin is more, flavonoid is more and phenol is more. While in Neem Leaf, alkaloid is less, tannin is abundant, saponin is less, flavonoid is more, then phenol is none. Tannin is predominantly rich in all the green leaf extracts.Deficiency of bioactive constituents in green plant extracts are often classified as negative or absent. For instance, Madhurima *et al*.,[4]study of phytochemical constituents on Calotropis gigantea, flavonoids were absent in the aqueous extraction, methanolic extraction and acetate extraction, while tannins and saponins were absent in both methanolic extraction and ethanolic extraction respectively. Also, Olanrewaju *et al*., [22] study on qualitative and quantitative evaluation of the phytochemicals in Morinda lucida, alkaloids were absent in the various studies.

3.2Quantitative phytochemical analysis

Table 2 shows the quantitative analysis of the green leaf extracts. The percentage present in the various green leaf extracts revealed that in Scent Leaf, alkaloid was 6.317, tannin was 4.416, saponin was nil, flavonoid was 5.22% and phenol was 0.34%. In Cassava Leaf, alkaloid was 12.34%, tannin was 3.82%, saponin was 1.48%, flavonoid was 2.72% and phenol was 0.63%. While in Neem Leaf, alkaloid was 10.07%, tannin was

6.73%, saponin was 3.24%, flavonoid was 3.45% and phenol was nil. The result shows that alkaloid had the highest percentage in all the green leaf extracts. Phenol had the lowest percentage in two leaf extracts and had none in one extract.

Alkaloids

Fig. 1 shows alkaloids constituent in the various green leaf inhibitors. It was revealed that Cassava Leaf had the highest alkaloid bioactive constituent, while Scent Leaf had the least Alkaloid constituent. Cassava Leaf having the highest constituent indicated that alkaloid might be very active in it which might probably exhibit most corrosion inhibition on Mild Steel.

Tannins

Fig. 2 shows that Neem Leaf had the highest percentage of tannin, while Cassava Leaf had the least percentage of the bioactive constituents. Neem Leaf possessing the highest bioactive compound indicate that probably, it will exhibit significant corrosion inhibition on Mild Steel C1026 in corrosive environment (s).

Saponins

Fig. 3 shows the percentage of saponin radical present in the various green leaf extracts. It disclosed that Neem Leaf had the highest percentage, while Cassava Leaf had the lowest percentage of the radical. However, the percentage of the Scent Leaf was not indicated on the figure because saponin was not present in it.

Flavonoids

Fig. 4 shows the percentage of flavonoid bioactive constituent present in the various green leaf extracts. It is evident that Neem Leaf had the highest percentage, while Cassava Leaf had the lowest percentage of the radical. However, the percentage of Scent Leaf was not indicated on the figure because saponin was not present in it.

Phenols

Percentage of phenol radical present in the various green leaf extracts from the investigation shows that Cassava Leaf was 0.63% Scent Leaf was 0.54% and Neem Leaf had none existence of phenol radical.

Table 1 Qualitative Method Analysis of Scent Leaf, Cassava Leaf and Neem Leaf [12]

Sample	Alkaloid	Tannin	Saponin	Flavonoid	Phenol
Scent Leaf	+ +	+ +	-	+	+
Cassava Leaf	+	+ + +	+ +	+ +	+ +
Neem Leaf	+	+ + +	+	+ +	-

KEY

+ = Less, + + = More, + + + = Abundant and **- = None**

Table 2 Quantitative Method Analysis of Scent Leaf, Cassava Leaf and Neem Leaf [12]

Extract	Scent Leaf	Cassava Leaf	Neem Leaf
Alkaloid	6.31 %	12.34%	10.07%
Tannin	4.41%	3.82%	6.73%
Saponin	-	1.48%	3.24%
Flavonoid	5.22%	2.72%	3.45%
Phenol	0.34%	0.63%	-

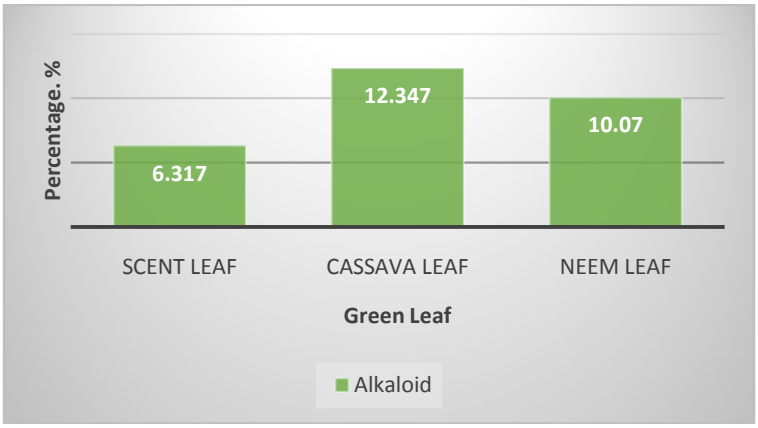

Figure 1 Phytochemical Quantitative of inhibitors with Alkaloid.

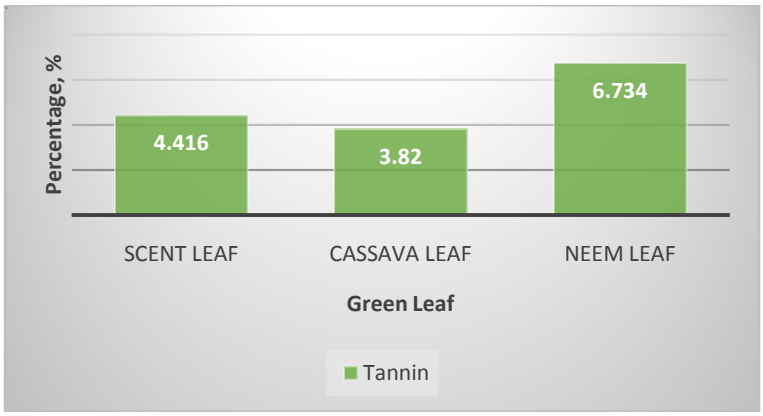

Figure 2 Phytochemical Quantitative of inhibitors with Tannin.

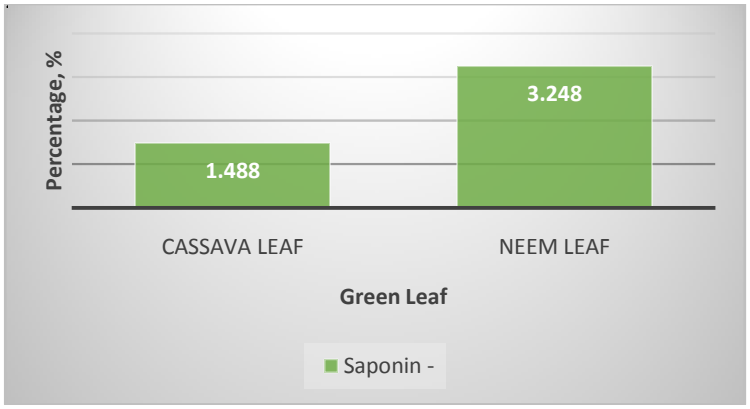

Figure 3 Phytochemical Quantitative of inhibitors with Saponin.

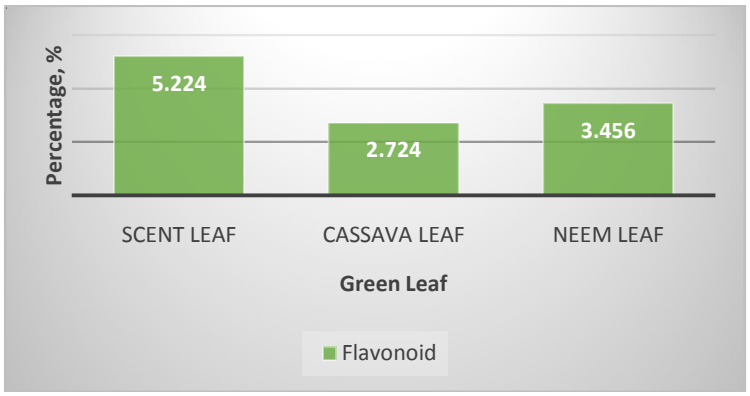

Figure 4 Phytochemical Quantitative of inhibitors with Flavonoid.

IV. DISCUSION

Secondary metabolite studies of green plant extracts of Scent Leaf, Cassava Leaf and Neem Leaf have shown the presence of alkaloid, tannin, saponin, flavonoid and phenol which are of great importance in corrosion inhibition of oil and gas pipeline and ethnomedical use for treatment and prevent of infections [23] in the field of research on the use of green plant as alternative to inorganic compounds. The green plant extracts studied are very much vital for therapeutic efficacy of drugs because of the secondary metabolites in them which compounds are similar with Soni et al. [24] study on qualitative and quantitative phytoconstituents in some herbs.

Quantitative test showed the presence of alkaloid, tannin, saponin, flavonoid and phenol.From the analysis, it was observed that alkaloid had the highest percentage in all the green plant extracts which was followed by tannin.

V. CONCLUSION

The qualitative and quantitative phytochemical evaluation of the green leaf extracts show that they are rich in alkaloids, tannins, saponins, flavonoids and phenols in Table 1 and 2. Fig. 1 – 4 shows the various trends of percentage of the chemical constituents in the plant extracts. Tannin is sufficiently abundant in Neem Leaf which may be responsible for its efficiency in the corrosion inhibition of Mild Steel by the green leaves in various corrosive environments.

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