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Comparative Growth Inhibitory Assay of the Methanol Extract of the Leaf and Seed of *Persea americana* Mill (Lauraceae)

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Persea americana is one of the recipes used by traditional healers in parts of Edo State of Nigeria for the treatment of cancer. This claim was investigated using predictive bench-top assay method of germinating seeds radicle of guinea corn (*Sorghum bicolor*). The powdered leaves and seeds were extracted by Soxhlet using methanol. The growth inhibitory effect was examined using radicle lengths of germinating seeds of *Sorghum bicolor* at 1-30 mg/ml. The methanol extract of the leaf of *Persea americana* was subjected to solvent partitioning. Phytochemical screening showed the presence of saponins, flavonoids, tannins and cardiac glycosides with no traces of anthracene derivatives. The aqueous fraction was more active than the chloroform fraction and completely inhibited the germination of the seeds at 20mg/ml. The results suggest the probable use of the plant in preparing recipes for tumor-related ailments. However, using appropriate human cell lines will further justify this claim

Keyword: *Persea americana*, Growth Inhibitory, Phytochemical, *Sorghum bicolor*

1. Introduction

The use of medicinal plants as sources of drugs in treating various forms of cancer diseases has attracted the attention of scientist worldwide. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. This is due to the fact that they are relatively cheap, safe and easily available compare to orthodox medicines. Since the beginning of life, man has depended on plants for food, drinks, shelter, clothing, equipment, dental care and medicine[1]. In fact, the early men depended on plants, herbs for treatment of various diseases before the introduction of orthodox medicine.

Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations[2]. Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack[3].

According to the WHO, 80% of the world's population, primarily those of developing countries rely on plant derived medicines for their healthcare[4]. More than a third of people in the developing countries have no access to essential medicines. Across the cultures, traditional medicine is mainly derived from plant. In deed about 25% of the modern drugs are derived from plants first used traditionally. The widespread use

of herbal medicine is mainly due to its ready availability and affordability.

The search for antitumor agents of natural origin continues to increase due to the non-selective pattern in the activities of the conventional drugs coupled with their high costs and scarcity, particularly in rural areas. Research into antitumor agents usually involves a series of complex procedures that sometimes produces non-encouraging results after much material and time has been expended. In addition to these, paucity of research funds has made the development and acceptance of simple bench-top assays imperative[5].

Ethnodicinal uses of plant are usually obtained from the direct users of the plants in the society most especially from traditional medical practioners. *Persea americana* have previously been reported to be used among the Owan people in Edo State in Nigeria in treating tumour-related ailments[6]. In local Nigerian languages, the plant is known as *Pia*-Yoruba, *Orumwu*-Bini, ube oyibo, Efik-*eban mbakara*[7] and *ewo ebo in* Owan speaking tribes of Edo State.

The plant *Persea americana* is a multipurpose used plant which has diverse applications in ethnomedicines ranging from treatment of diarrhoea, dysentery, toothache, intestinal parasites to the area of skin treatment and beautification[8]. It has also been reported to be used as an emmenagogue (hot water extract, decoction), for asthma (bark), used for cough, fever, kidney and liver troubles, for diabetes, as food, for skin blemishes, as an abortifacient, for female disorders among others[9].

2. Materials and Methods

The leaves and seeds of *Persea americana* were collected in March 2009 at a playground at Okhoro, a suburb in Benin City. The identity of the plant was confirmed by Dr. Shasanya Olufemi, the Plant Taxonomist at the Forest Research Institute of Nigeria (FRIN), Ibadan. A herbarium specimen number FHI 109574 was deposited at the institute for reference. The plant material was air dried in the laboratory for 5 days at room temperature followed by oven drying at 40° C followed by grinding to powder form using an electric mill. The powdered sample was kept in an air tight container until required.

2.1 Extraction of the Plant Material

1kg of the powdered leaves and seeds were extracted by Soxhlet extractor using methanol in 2.5 L of methanol. The liquid extracts obtained were concentrated using a rotary evaporator maintained at 40°C and were kept in the refrigerator.

2.2 Phytochemical Screening of the Plant Material

This was carried out to test for the presence or otherwise of tannins, flavonoids, saponins, alkaloids, steroidal glycosides and anthracene derivatives using standard methods[10,11].

2.3 Source and Preparation of the Guinea Corn *Sorghum bicolor*

Seeds of *Sorghum bicolor* (Guinea corn) were purchased from Oba market in Benin City. A simple viability test was carried out by placing a handful of the seeds in distilled water. The viability of the seeds was determined by their ability to remain submerged in water. The viable seeds were washed with 95% ethanol for sterilization for 1 minute and were finally rinsed with distilled water and dried for use.

2.4 Determination of The Growth Inhibitory Effects of the Extracts on Guinea Corn (*Sorghum bicolor*)

10 ml different concentrations of the leaf methanol extract (1-30 mg/ml) containing 5% DMSO was poured into the petri-dish of about 9cm wide containing filter (Whatman No.1) underlay with cotton wool, after which twenty of the sterilized seeds were spread on each of the petri-dishes. The petri-dishes were incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured at 24, 48, 72 and 96 hours. The control seeds were treated with 10ml distilled water containing 5% DMSO (*13*).The experiment was carried out in triplicates for all concentrations and controls while the radicle lengths were measured to the nearest millimetre.

The procedure was repeated for the seed methanol extract.

2.5 Partitioning of the Methanol Extract of the Leaf of *Persea americana*

97g of the crude methanol extract of the leaves were re-dissolved in methanol-water (1:1) and partitioned exhaustively with chloroform (200 ml ×4) volumes in a separating funnel. The aqueous and the chloroform fractions were concentrated to dryness on a rotary evaporator and their respective yields noted. The growth inhibitory effects of the two fractions were separately carried out as earlier described above.

3. Statistical Analysis

All data were expressed as mean ± SEM and one way Analysis of Variance Anova statistical test using Graph pad Instant R version 2.05 (UK) was used to test for significance. P< 0.05 was considered Significant.

4. Result

The 2kg of the powdered leaf and seeds of *Persea americana* were observed to yield **95.55** and 76.32 g of the methanol extracts corresponding to 4.78 and 3.82 % respectively.

Partioning of the methanol extract of the leaf gave 42.57g (2.13%) of the aqueous and 28.55g (1.43%) of the chloroform fraction respectively.

The leaf and seeds of *Persea americana* were observed to contain alkaloids, saponins, tannins, flavonoids, tannins and cardiac glycosides while anthracene derivatives were absent (Table 1).

Table 1: The results of the preliminary phytochemical screening of the methanol extracts of the leaves and seeds of *Persea americana.*

CONSTITUENTS	LEAF EXTRACT	SEED EXTRACT
Alkaloids	++	+
Anthraquinone	-	-
Cardiac glycosides	+++	+
Flavonoids	+++	+
Saponin	+++	++
Steroids	+	+
Tannins	+++	+
Terpenoids	+	+

- = not detect; + = low; ++ = moderate; and +++ = strong.

4.1 Results of the Extracts on Radicle Growth.

The results of the growth inhibitory effects of the extracts of the plant parts on guinea radicle length showed a concentration dependent reduction in length of the radicle that emerged from the guinea corn seeds. At 24 h, control seeds showed an average radicle length of 4.61±0.99 mm compared to average length of 0.65 ± 0.27 mm(86% inhibition) shown by the seeds pre-treated with 20mg/ml of the leaf extract. Almost complete inhibition of germination (100% inhibition) was observed in seeds pre-treated with 30 mg/ml of the extract. After 96 hour incubation period, the control seeds produced an average length of 34.72 7.37 mm whereas the seeds pre-treated with 20 and 30 mg/ml of the extract produced average lengths of 1.73 ± 0.67 and 1.38 ± 0.66 mm respectively (Fig. 1). These implied 95% and 97% reduction in length compared to the control seeds. These variations in length were found to be significant at P<0.01

Similarly for the seed extract, the control seed at 24 hr gave an average length of 3.87 ± 0.59 mm compared to 1.63 ± 0.29 mm (57.88 %

inhibition) and 0.67 ± 0.03 mm (82.69 % inhibition) showed by seeds pre-treated with 20 and 30 mg/ml of the seed extract. Also, at 96 hr an average length of 61.85 ± 7.83 mm was recorded for the control seeds while 8.62 ± 1.92 mm (86.06 % inhibition) and 6.25 ± 1.44 mm (89.89 % inhibition) as shown in Fig. 2. These variations in length were found to be significant at $P<0.01$

The aqueous and the chloroform fractions of the methanol extract of the leaf were also observed to suppress the growth of the radicle length with increase in concentrations with the aqueous fraction observed to be a little more effective than the chloroform fraction. At 24 hours, the average radicle length of the control seeds was 5.00 ± 0.77 mm while seeds pre-treated with 2, 5 and 10mg/ml of the aqueous fraction produced average lengths of 2.97 ± 0.31, 2.32 ± 0.51, and 0.9 ± 0.06 mm which implies 41, 54 and 87 % reduction in radicle length compare to the control seeds. Those pre-treated with 20 and 30 mg/ml concentrations produced no signs of germination. After 96 hours, the control had an average length of 47.07 ± 6.05 mm while seeds pre-treated with 2, 5 and 10mg/ml showed an average lengths of 16.52 ± 2.84mm (65 % inhibition) 10.85 ±1.67 mm (77 % inhibition) and 2.92 ± 0.52 mm (94 % inhibition) respectively. Throughout the period of incubation, 20 and 30mg/ml completely inhibited the germination of the seeds (Fig. 3). The variations in the growth inhibition were observed to be significant at $P<0.05$

The chloroform fraction also produced remarkable inhibition of the radicle growth but germination of seeds and hence radicle growth were not absolutely inhibited as seeds pre-treated with 20 and 30 mg/ml of the fraction produced 0.38 ± 0.08 and 0.15 ± 0.03 mm respectively compared to 54.97mm produced by the control after 96 h incubation period (Fig. 3).

5. Discussion

The medicinal value of any plant is measured by the extent to which it is able to remove or mitigate the harmful effects of diseases or organisms causing them. The therapeutic effect of such plant is also a direct function of the various constituents it contains naturally, which may be acting synergistically with one another. Close examination of the results of the phytochemical screening of the two morphological parts used showed that the various groups of phytochemical constituents such as reducing sugars, saponins, alkaloids, tannins, flavonoids, cardiac glycosides were more on the leaf but fairly distributed in the seeds.

The activity of the leaf extract was remarkably pronounced in the inhibition of the growth of radicles of *Sorghum bicolor* seeds. At 30 mg/ml, the extract produced an average length of 1.38 ± 0.66 mm at 96 hr of incubation period while the seeds produced 6.25 ± 1.44 mm. These observations which obviated the choice of the leaf extract for further work implied that the constituents responsible for the activities were mostly concentrated in the leaves. It could be that the constituents were biosynthesized in the leaves, after which some quantities are latter translocated to the other parts of the plants including the seeds.

The use of guinea corn seeds (*Sorghum bicolor*) was necessitated by the fact that meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions and the extent of proliferation is reflected in the increase in the length of the radicles produced in 96 hrs in the control seeds. Although any seeds such as cowpea (*Vigna unguiculata*), maize (*Zea mays*) and other seeds can be used, that of *S. bicolor* was found to be most convenient because of its relatively small size. The experiment can be carried out in plates or in small glass containers; hence the amount of solution required is small. Also, the availability is high and up to 90% can germinate within 24hours.

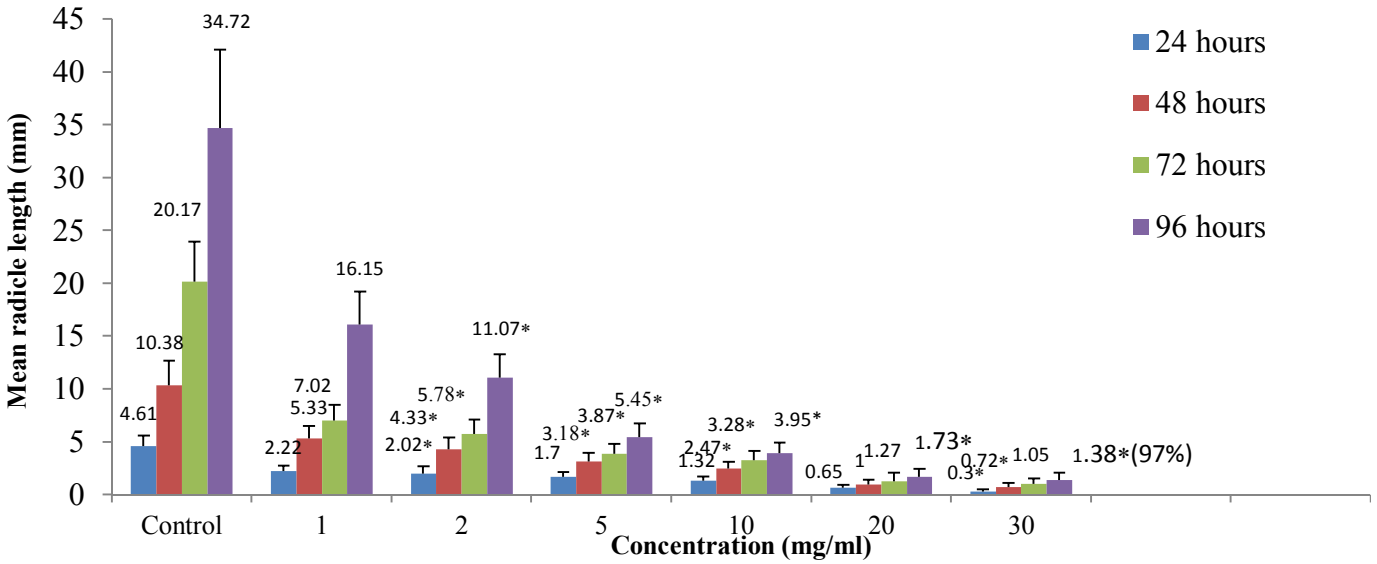

Fig. 1: The growth inhibitory effects of the methanol extract of the leaf of *P. americana* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20. ∗Significantly different from control. P<0.05

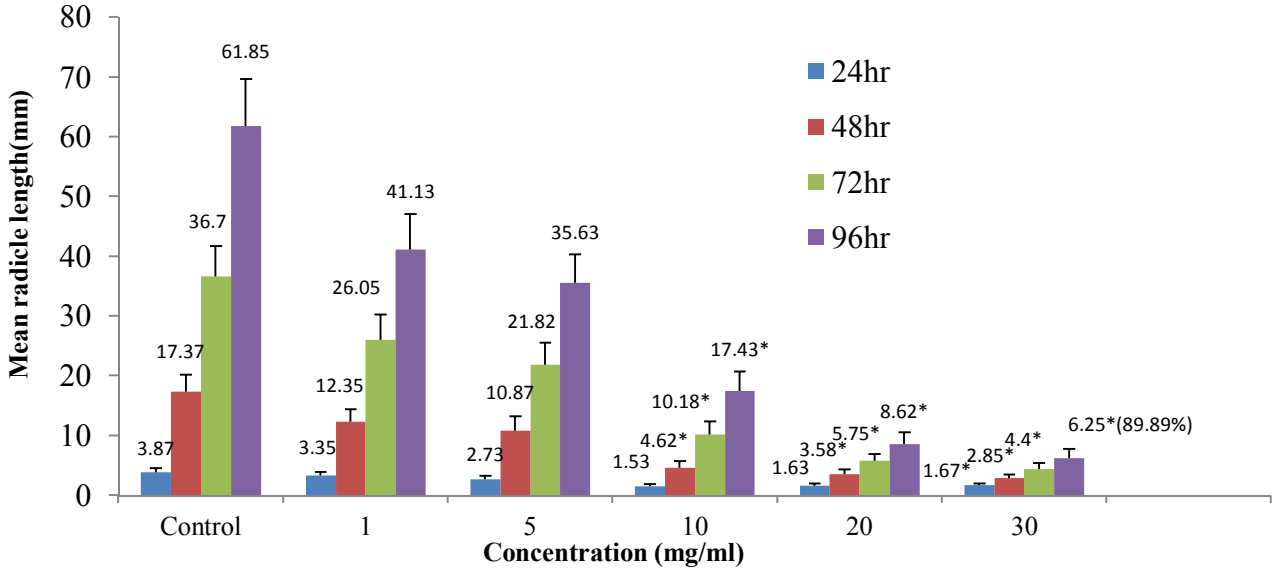

Fig. 2: The growth inhibitory effects of the methanol extract of the seeds of *P. americana* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.∗Significantly different from control. P<0.05

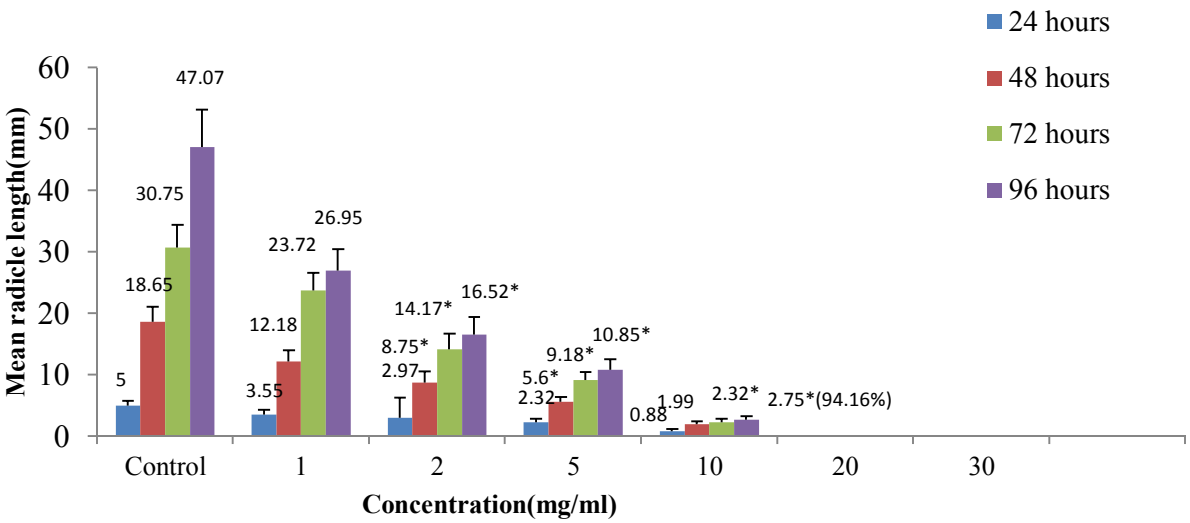

Fig 3:Inhibitory effects of the aqueous fraction on guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

*Significantly different from control.P<0.05

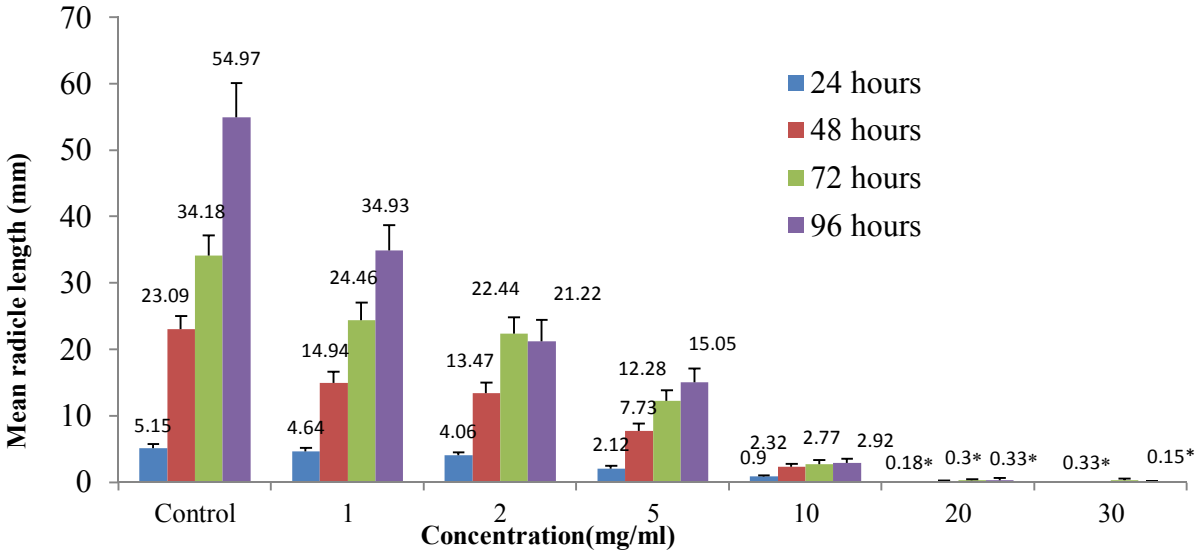

Fig 4: Inhibitory effects of the chloroform fraction on the growth of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

*Significantly different from control. P<0.05

As plants are known to contain many constituents of varying polarities, molecular mass and concentrations, using two appropriate immiscible solvents ensures the separation of the constituents based on their relative solubilities in the solvents. As stated earlier, the choice of methanol extract of the leaf for partitioning was based on the higher activities it exhibited over the seeds.

Partitioning of leaf extracts into chloroform and aqueous phases remarkably improved the growth inhibitory effects of the constituents. For instance, the aqueous fraction completely inhibited seed germination and subsequent growth of the guinea radicle at 20 mg/ml compared with the 30 mg/ml of the extract which produced 1.38 ±0.66 mm at the end of 96 h incubation period. For the aqueous fraction, it is possible that some of the constituents may have affected water and consequently osmotic potential thus preventing the development of turgor pressure in the seed, which has been considered as one of the key factors required for the initiation of radicle growth during seed germination[12]. This goes to show that the constituents responsible for this effect are polar in nature.

Comparing the activities of *Persea americana* with other plants like *Struchium sparganophora*, it was observed that the latter is more potent than the former. The methanol extract of *S. sparganophora* at 4mg/ml reduces the growth of radicle to 47.16 % compare to the control while the aqueous and chloroform fractions reduces the growth of radicle to 43.81 and 32.51% % respectively[13].

From this study, it can be inferred that *Persea americana* leaf may likely have effects on tumour-producing cells as claimed in ethnomedical uses of the plant among the Owan people of Edo State. However, further investigation using tumour cell lines *in vitro* or *in vivo* may be necessary to confirm the folkloric anti-tumour activity of *Persea americana.*

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