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Isolation and Purification of Flavonoids from the Leaves of *Mitracarpushirtus* Plant

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Abstract: *n- Hexane defatted leaf of Mitracarpushirtus was extracted with Methanol. The extracts were screened for the active components present. The N-hexane extract showed the presence of Flavonoids, Alkaloids, Saponin, Tannin, Glycoside, Anthraquinone, Resins and Steroids while the Methanolic extract showed the presence of Flavonoids, Saponin and Tannin. Methanolic extract (6g) was chromatographed. The flavonoid fraction was isolated using Column Chromatography over Silica gel Column (230-400 mesh) and eluted with the solvent mixture of $CH_3C1/CH_3OH/H_2O$ in the ratio (70:30:1 V/V). The Flavonoid fraction collected was purified using re-crystallization method and a yield of 17.90% was obtained.*
Key Word: *Isolation, Flavonoids, Mitracarpushirtus*

I. Introduction

Mitracarpushirtus PLANT

Mitracarpushirtus is a small plant that grows up to 40cm tall as a shrub. It is an ornamental plant that grows as a weed around plantations and crops. It is an annual plant that is widely used in folk medicine in tropical Africa, America, India, China and Australia.Okoye et al (2013) reported that the leaf of the plant is well known for its use in the treatment of skin diseases. It is highly used in the treatment of so many diseases due to its antimicrobial activities

The Polyphones

Polyphenols are products of the secondary metabolism in plants. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins (Harborne, 1980). Polyphenols serve as antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity (Del Rio *et al*, 1997).

Favonoid is a secondary metabolite compound that has a diverse biological activities ranging from toxicity to hormonal mimicry, and may play a role in protecting plants from herbivory and disease (Hagerman, 2002). Flavonoids have great potentials that can be invaluably applied in the Chemistry of Natural Products.

Natural Products

The following components of natural products: Alkaloids, Saponins, Steroids, Flavonoids, Lipids, Carbohydrates, Tannins, Proteins and Vitamins etc, have many effects on the animal system and some processes that have important therapeutic properties, which can be utilized in the treatment and cure of human and other animal diseases such as hypertension. Drug discovery from natural products can be an engine for such conservation and sustainable economic development in non industrialised countries that are strapped for cash but rich in natural resources (David and Emma (2011).

The aim of this research work is to isolate Flavonoids from the leaves of *Mitracarpushirtus* plant as a source of non-nutritive plant chemicals that have protective, antioxidative or disease preventive properties.

II. Materials And Methods

Materials

The leaf of Mitracarpushirtus was collected from Bayara Village in Bauchi state of Nigeria. The Plant was identifiedas *Mitracarpushirtus* plantby a botanist. The leaves were dried under shade for 3 days, grounded into fine powder and sieved using a laboratory test sieve of 212mm aperture.

Extraction

The fine powder of *Mitracarpushirtus* (30g) was defatted with N-Hexane (250ml) using Soxhlet extraction. The extraction was carried out for about six hours at a temperature between 65^{o}C and 70^{o}C. The defatted marc was further extracted with Methanol (250ml) at 60^{o}C and the solvent in the extract was evaporated on a waterbath.

Phytochemical Screening
The *Mitracarpushirtus* plant extracts were phytochemically screened for the secondary metabolites.

Test for Flavonoids (Herbone, 1973)
The plant extract (2ml) was acidified with 1% HC1 and dissolved in 20% NaOH and observed for a canary yellow colour which indicates the presence of Flavonoids.

Test for Saponins (Trease and Evans, 1989)
The presence of Saponins in the leaf extracts of *Mitracarpushirtus* was tested by boiling 2g of the extract with 20ml of distilled water in a bath. It was then filtered using a filter paper. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. 3 drops of olive oil was then added to the froth and then shaken vigorously again. The formation of an emulsion indicated the presence of Saponins.

Test for Tannins (Sofowora, 1984)
2ml of the extract was treated with two drops of 5% $FeC1_3$. The formation of a green precipitate on dilution indicates the presence of Tannins.

Test for Steroids (Herbone, 1973)
Sakowski's test: 2ml of concentrated H_2SO_4 was added to 2ml fraction of the *Mitracarpushirtus extract*. Appearance of effervescence after which a clear reddish brown colour appeared at the interface confirms the presence of steroids.

Test for Anthraquinone (Ciulei, 1975)
2ml of each extract was treated with 5ml of benzene and observed for two layers. The clear colourless upper layer was pipette and the organic layer was treated with 3ml of 10% NH_3 so as to observe a change in colour from rose pink to red.

Test for Cardiac Glycoside (Sofowora, 1984)
2ml of each extract was added in succession to 3ml, 3.5% Iron (III) Chloride, then 3ml Ethanoic acid and observed for a green precipitate and dark coloured solution respectively.

Test for Resin (Trease and Evans, 1995)
2.0g f each extract was dissolved in 10ml of Ethanoic acid anhydride. One drop of concentrated H_2SO_4 was added and observed for a purple colour which rapidly changes to violet.

Test for Alkaloids (Treaseamd Evans, 1989)
2g leaf extracts were warmed with 20ml of 1% Tetraoxosulphate (VI) acid in conical flask on a water bath for 2 minutes. It was intermittently shaken and centrifuged to obtain the supernatant. A drop of Meyer's reagent was added to 0.1ml of the supernatnant in a test tube and observed for a cream precipitate.

Isolation of Flavonoids By Column Chromatographic Method
Methanolic extract (6g) was chromatographed over Silica gel Column (200g: 230-400 mesh) and eluted with the solvent mixture of $CH_3C1/CH_3OH/H_2O$ (70:30:1. V/V). The Flavonoid fraction was then purified using the recrystallization method.

III. Results And Discussions

The n-hexane extract contain more components than the Methanol extract as in the results for the phytochemical screening shown in table 1. The results for phytochemical screening of the extracts isolated from the Column Chromatography are shown in table 2.

Table 1: Results for the Phytochemical Screening

Components	N-Hexane extract	Methanol extract
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Anthraquinone	+	-
Steroid	+	-
Alkaloid	+	-
Glycoside	+	-
Resins	-	-

Hints: "+" indicates presence, "-"indicates absence

Table 3: Phytochemical screening of the isolated extracts from Column Chromatography

Component	Flavonoid
Fraction 1	-
Fraction 2	+
Fraction 3	-

Hints: "+" indicates presence, "-"indicates absence

IV. Discussion

N-Hexane extracts showed the presence of the following active ingredients: Flavonoids, Tannin, Saponin, Steroids, Anthraquinone, Alkaloids, Resins and Glycosides. The Methanolic extract showed the presence of Flavonoids, Tannin, and Saponin. The three different components were isolated through the column chromatography where one of the components gave the Flavonoid and after recrystallization a yield of 17.90% was obtained.

V. Conclusion

The Mitracarpushirtus is a good source of Flavonoids which has an important antioxidant properties.

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