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PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITY OF CRUDE  
METHANOL LEAF EXTRACT OF *RAPHANUS RAPHANISTRUM* L.  
(BRASSICACEAE)

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**ABSTRACT**

*Raphanus raphanistrum* is a rapidly growing, cool-season annual crop cultivated in Jos, Plateau State, Nigeria. Originally native to Europe and Asia, this plant belongs to the Brassicaceae family. Fresh leaves were harvested from mature white radish plants in Jos for this study. *Raphanus raphanistrum* can grow as either an annual or biennial plant. Preliminary phytochemical analysis of the Methanol leaf extract revealed the presence of various bioactive compounds, including alkaloids, anthraquinones, cardiac glycosides, flavonoids, and tannins. Conversely, the blood glucose levels in extract treated albino rats at the dose of 800 mg/kg after 8 and 24 hours of administration was found to increase. This shows that the plant extract at the dose of 800 mg/kg has lost its hypoglycemic effect compared to others doses used. The Methanol Leaf extract of *Raphanus raphanistrum* demonstrated a moderate antidiabetic effect which was not dose dependent even though the activity is not comparable to that of the standard drug used (insulin).

**Keywords:** *Raphanus raphanistrum*, bioactive compounds, phytochemical constituents, antidiabetic activity

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**INTRODUCTION**

Medicinal activities of plants have long been associated with the production of secondary metabolites which include alkaloids, flavonoids, tannins, terpenoids, coumarins, etc. Phytochemicals are bioactive chemicals synthesized naturally in all parts of the plant body. They are called secondary metabolites because the plants that produce them may have little need for them. Secondary metabolites are known to show curative activity against several ailments in man and other animal. Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action or both (Jayakar

and Suresh, 2003). These products help plant to carry out various activities like defense and pollination. However, their antidebetic, antioxidant, antimicrobial and other medicinal properties are widely exploited for the benefit of mankind regarding healthcare. Certain biological assays are conducted in order to assess the phytochemicals and antimicrobial potentials of a plant. (Lugasi., *et al.*, 2005). Herbal products are suitable for treating a wide range of infections and diseases.

*Raphanus raphanistrum* is originally from Europe and Asia. It belongs to the Brassicaceae family. It is an annual or biennial plant. It grows in temperate climates at altitudes between 190 and 1240

m. It is 30-90 cm high and its roots are thick and of various sizes, forms, and colors. They are edible with a pungent taste. Salted radish roots which are consumed in the amount of about 500,000 tons/year in Japan, are essentially one of the traditional Japanese foods. The salted radish roots have a characteristic yellow color, which generates during storage. This specie is used popularly to treat liver and respiratory illnesses (Paredes. 1984).

The antibiotic activity of its extracts and its time persistence validates its effectiveness in microbial sickness as reported in traditional medicine. The roots juice showed antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Salmonella thyphi*.

The Methanol and aqueous extracts showed activity against *Streptococcus mutans* and *Candida albicans*. Aqueous extract of the whole plant presents activity against *Sarcinia lutea* and *Staphylococcus epidermidis* (Caceres.1987). Aqueous extract of the leaves showed antiviral effect against influenza virus. Aqueous extract of the roots showed antimutagenic activity against *Salmonella typhimurium*. In this review, the metabolites produced by *R. sativus* are presented according to structural classes. The main season for sowing the radish in north Indian plains is mainly from September to March, while in the hills they are sown from March to July. This crop is harvested within 30-50 days of sowing and is pulled out from the soil when it reaches edible size. The ancient inhabitants of Greek prized radishes above all root crops. The root crop was a common food in Egypt long before the pyramids were built, and was popular in ancient Rome as well. The word "radish" is a derivation of the Latin word "radix," or root. Columbus and the

early settlers brought radishes to America. Radish can sprout from seed to small plant in as little as 3 days (Jones *et al.*, 1982).

The portion that is eaten from radish is root and leaves. The radish has a special pungent taste due to it containing mustard oil. There are many varieties of radish, including white radish, red radish, radish black, and many others (Nakamura *et al.*, 2008). Radish plant is rich in vitamin C and vitamin A and contains many mineral elements including iodine, sulfur, Calcium, and Potassium. So, radish is plant of high nutritional value as well as being appetizing and digestion. Radish plants (roots, leaves and seeds) have many medical uses, including treatment of tuberculosis, whooping cough, asthma attacks, laxative to treat constipation, Fracture of kidney and bladder stones, thinner, cholesterol-lowering, antiradical and analgesic pain (joint medical problems affecting the joints and textile) (Gutierrez *et al.*,2007). Radish plant is useful in treating diarrhea (Al-Thwani *et al.*, 2010). Radish root and leaves contain many active compounds, including coumarins, alkaloids, nitrogenous compounds, gibberellins, glycosylate, organic acids, Phenolic compounds, and sulfur pigments (Perez and Rosalinda, 2004). It contains antioxidant compounds (Lugasi *et al.*, 2005). Radish root and leaf extracts are effective against antimicrobial microorganisms because of their effective secondary compounds. Radish plant extract is used to treat viral diseases, including those caused by the Herps virus (Weilan *et al.*, 1987) which are caused by mononucleosis, chickenpox (Varicella, chickenpox), skin rash in infants. Radish contains antioxidants that protect the human body from anti-cancer and these include cytokines and other antioxidants

Harborne and Baxter, 1993). That is, horseradish protects the body from cancer (Kim *et al.*, 2011). Radish plant extract inhibits cell division (Beevi *et al.*, 2009; Papi *et al.*, 2008; Yamasaki *et al.*, 2009) for containing antioxidants. People with diabetes are advised to eat radish because of its effect in lowering blood sugar levels (Shukla *et al.*, 2011). Radish plant extract is used to treat constipation because it contains some of the compounds that stimulate bowel activity (Yong *et al.*, 2000). Evaluating the efficacy of radish extract in controlling insects and inhibiting germination of seeds of some plants.

## **MATERIALS AND METHODS**

### **Collection and Preparation of Plant Materials**

Fresh leaves of *Raphanus raphanistrum* was collected from Jos, Plateau state from matured white radish plants on the 10<sup>th</sup> January, 2024. The plant was then authenticated by Dr. Cletus A. Ukwubile a plant Taxonomist of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri. A voucher specimen number (UMM/FPH/BRC/001) was assigned and sample deposited in the Research Laboratory of the Department of Pharmacognosy, Faculty of Pharmacy University of Maiduguri, Borno state, Nigeria. The leaves were air-dried and pulverized to a fine powder and kept at room temperature until used.

### **Extraction of the Plant Material**

Using analytical weighing balance 800 g of the dried powdered material was successively weighed and extracted differently with the following solvents; *n*-hexane, ethyl acetate, Methanol and Methanol.

The process of the extraction was cold maceration for 72 hours in accordance to

the increasing order of polarity of the solvents. The filtrate was obtained for each solvent and evaporated to dryness 45°C, and the marc was then discarded. (Mohammed, 2013).

### **Preliminary Phytochemical Screening**

The extracts were screened for the presence of primary and secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedure described by (Trease and Evans, 2002).

#### **Test for Carbohydrate**

i. Molisch's test: Four grammes (4 g) of the extract was dissolved in distilled water, the mixture was filtered with a filter paper, a few drops of molisch's reagent were added to 2 mL of the extract, then 1ML of concentrated sulphuric acid was allowed to run down the inclined tube to form a layer, the mixture was allowed to stand for two minutes and then diluted with 5 mL of distilled water. A purple colour observed at the interface indicate the presence of carbohydrate (Trease and Evans, 2002).

ii. Barfoed's test: One millilitre of Barfoed's reagent was added to 2 mL of the extract in a test tube and heated in a water bath for 2 minutes. A red precipitate of cuprous oxide indicated the presence of monosaccharides like glucose or fructose (Trease and Evans, 2002).

iii. Fehling's test: the extract was dissolved in 2 mL distilled water and filtered. The filtrate was heated with 5 mL of equal volumes of Fehling's solution A and B, the formation of brick-red cuprous oxide precipitate indicates the presence of reducing sugar like glucose or fructose (Trease and Evans, 2002).

#### **Test for Glycosides**

i. Liebermann-Burchard test: Two

millilitre (2 mL) of acetic anhydride was added to 0.5g of the extract and then well cooled in ice, then concentrated sulphuric acid was carefully added, development of violet to blue-black indicated the presence of steroid i.e. the aglycone portion of cardiac glycoside (Sofowora, 2008).

- ii. Salkowski's test: About 0.5g of the extract was dissolved in 2 mL of chloroform in a test tube. Concentrated sulphuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish-brown colour at the interface indicated the presence of the glycoside (Sofowora, 2008).

#### **Test for Anthraquinones**

- i. Borntrager's test: the extract of the plant material was shaken vigorously with 10ML of benzene, filtered and 5 mL of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free anthraquinones (Trease and Evans 2002)

#### **Test for Saponins**

Froth test: Exactly (0.5g) of the extracts was dissolved in 10 mL of distilled water in a test tube and was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over 30 minutes. Honey comb froth above the surface of the liquid persists after 30 minutes, the sample is suspected to contain saponins (Sofowora, 2008).

#### **Test for Flavonoids**

- i. Ferric chloride test: the extract was boiled with distilled water and

filtered; a few drops of 10% ferric chloride solution was added. The appearance of a green blue or violet colour indicated the presence of phenolic hydroxyl group (Trease and Evans, 2002).

- ii. Sodium hydroxide test: dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water, the mixture was inspected for the production of yellow colour which is considered as positive test for flavonoids (Trease and Evans, 2002).

#### **Test for Tannins**

Ferric chloride test: a portion of the extract were dissolved in water, the solution was clarified by filtration, 10% ferric chloride was added to clear filtrate. This was observed for a change in colour to bluish-black which indicated the presence of tannins (Trease and Evans, 2002).

#### **Test for Alkaloids**

About 0.5g extract was stirred with 5 mL of 10% aqueous hydrochloric acid on a water bath and then filtered. 3.5ML of the filtrate was taken and divided into three portions in the test tube 1ML each.

- i. Dragendoff's test: Three (3) drops of Dragendoff's reagent were added to the first portion. The formation of an orange-red precipitate indicated the presence of alkaloids.
- ii. Mayer's test: Three drops of Mayer's reagent were added to the second portion. The formation of a buff-coloured precipitate indicated the presence of alkaloids.
- iii. Wagner's test: Three drops of Wagner's reagent were added to the last portion. The formation of a dark-brown precipitate indicated the presence of alkaloid (Trease and

Evans, 2002).

### Thin Layer Chromatography

The thin layer chromatography of the leaf extract of *Raphanus raphanistrum* (n-hexane, ethyl acetate, Methanol and Methanol) fraction was carried out using prepared TLC plates in the developing chamber, 90% Methanol was added in adequate amount and set aside, to the different extracts, Methanol was added to enable spotting. The TLC plates was labelled to contain each extract to be spotted and then carefully inserted in the developing chamber. After then, it was then removed, sprayed with sulphuric acid and heated in an oven. The distance travelled by the compounds was then recorded.

### Experimental Animals

A total of 90 apparently healthy Wistar albino rats (150-170 g) of both sexes were used for the study. The rats were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, and were checked out for signs of illness and anomalies. Animals were acclimatized to the laboratory environment for two weeks before they were used for this study. During the experiment, the rats were housed under controlled environmental condition at room temperature and had free access to food and water *ad libitum*. The rats were handled according to CIOMS and ICLAS, 2012; the guideline for international guiding principles for biomedical research involving animals.

### Acute Toxicity

Modified Lorke's method was used to determine the acute toxicity (LD<sub>50</sub>) of the Methanol leaf extract of *Raphanus raphanistrum*. Three different doses of the Methanol leaf extract of *Raphanus*

*raphanistrum* was administered orally to the rats in both phases 1 and 2. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsion, and mortality for 24 hours. Two rats were used in each of the groups in both phases.

#### Phase I

- Group 1. Methanol leaf extract of *R. raphanistrum* (10 mg/kg body weight).
- Group 2. Methanol leaf extract of *R. raphanistrum* (100 mg/kg body weight).
- Group 3. Methanol leaf extract of *R. raphanistrum* (1000 mg/kg body weight).

#### Phase II

- Group 1. Methanol extract of *R. raphanistrum* (1600 mg/kg body weight).
- Group 2. Methanol extract of *R. raphanistrum* (2500 mg/kg body weight).
- Group 3. Methanol extract of *R. raphanistrum* (5000 mg/kg body weight).

### Pilot Study and Induction of Experimental Diabetes in Wistar Strain Albino Rats

Based on the method described by Ihedioha *et al.* (2014), 150 and 160 mg/kg body weight of freshly prepared alloxan monohydrate given intraperitoneally is used to induce experimental Type I diabetes in rats but according to Jain and Arya (2011), the dose of alloxan required for inducing diabetes depends on the animal species and route of administration and it is better to standardize the dose first because of inconsistencies associated with alloxan-induced experimental diabetes in animals.

Therefore, in accordance with this, a pilot study was conducted to select an appropriate dose to be used. Three doses of alloxan monohydrates; 150, 160 and 170 mg/kg body weight were used for the pilot study. Four groups of two rats were used, group 1 served as normal control while groups 2, 3 and 4 for 150, 160 and 170 mg/kg body weight of alloxan monohydrates respectively. The animals were fasted overnight prior to induction and each of the three groups was given different doses of freshly prepared alloxan as follows 150, 160 and 170 mg/kg body weight and after 24 hours post administration, diabetes was induced by a single intraperitoneal injection of alloxan monohydrate through the confirmation of fasting blood glucose levels above 200 mg/dl.

After the pilot study, 150 mg/kg dose of alloxan monohydrate was found to induce diabetes in the experimental rats with a consistent increase in blood glucose level and subsequently was used for the study. Thus, for induction of diabetes to assess the antidiabetic effect of *R. raphanistrum*, the rats were allowed to fast for 18 hours and freshly prepared Alloxan in normal saline at room temperature was administered immediately intra-peritoneally to rats with the exception of the normal control group which was given distilled water. After 24 and 48 hours of alloxan administration, the blood glucose levels of the rats were determined respectively using an Accu-chek glucometer based on the glucose oxidase method (ADA, 2003). Rats with blood glucose level of 160 mg/dL and above (maximum 600mg/dl) was considered diabetic and selected for the study (Ihedioha *et al.*, 2014)

### **Determination of the Antidiabetic Effects of *R. raphanistrum* Methanol Leaf Extract**

A total of 35 rats were randomly divided into seven groups of five rats each. Group 1 served as normal control and was given normal saline. Group 2 served as diabetic control with no treatment. Group 3 served as positive control and injected intraperitoneally with soluble insulin (positive control) while groups 4, 5, 6 and 7 were treated orally with four different doses (200, 400, 800 and 1600 mg/kg body weight respectively) of Methanol Leaf Extract of *R. raphanistrum* obtained from one third of the lethal dose LD<sub>50</sub> of *R. raphanistrum* which was found to be greater than 5000mg/kg body weight.

- Group 1. Normoglycemic + distilled water (normal control).
- Group 2. Diabetic untreated (diabetic control).
- Group 3. Diabetic + regular insulin (0.75IU/kg).
- Group 4. Diabetic + Methanol extract (200 mg/kg).
- Group 5. Diabetic + Methanol extract (400 mg/kg).
- Group 6. Diabetic + Methanol extract (800 mg/kg).
- Group 7. Diabetic + Methanol extract (1600 mg/kg).

### **RESULTS**

In this experiment, 800 g of the resultant size reduced leaf powder of *Raphanus raphanistrum* was used. The Methanol extract showed the highest yield of 32 g, (with percentage yield of 4%). The percentage yield is given in the table below.

**Table 4.1: Percentage Yield and Colour of Methanol Leaf Extract of *Raphanus raphanistrum***

Fraction	Wight (g)	Yield (%)	Colour
Methanol	32	4.0	Brown

**Phytochemical Screening of *Raphanus raphanistrum***

Table 4.2 below shows the phytochemical constituents of n-hexane, ethyl acetate,

Methanol, Methanol extracts of the leaf of *Raphanus raphanistrum*. The screening was conducted under standard procedure as described by Trease and Evans, (2002) using various reagents.

**Table 4.2: Phytochemical Constituents of Methanol Leaf Extracts of *Raphanus raphanistrum***

Constituents	Test	n-Hex	EtOAc	Methanol
Carbohydrate	Molish's	+	+	+
Tannins	Ferric Chloride	+	-	+
	Lead acetate	+	-	+
Glycoside	Keller-Kiliani	+	+	+
Saponins	Frothing	-	-	-
Alkaloids	Dragendoff	+	+	+
	Mayer's	+	+	+
Anthraquinones	Borntrager's	+	-	+
Flavonoids	Sodium Hydroxide	+	+	+
	Shinoda	+	+	+

**KEY :** + = positive - = negative n-Hex = Hexane, EtOAc =Ethyl acetate

The result obtained for thin layer chromatography is as follows

Length of plate = 7cm

Solvent front = 3.5cm

The movement of the spot were expressed by its retardation factor ( $R_f$ )

$$R_f = \frac{\text{distance traveled by solute}}{\text{distance traveled by solvent}}$$

Table 4.3 below shows the result using 100% chloroform as the solvent system for the thin layer chromatography of leaf extract of *Raphanus raphanistrum*.

**Table 4.3 Thin Layer Chromatographic Result of Methanol Leaf Extract of *Raphanus raphanistrum***

Extracts	Distance traveled by solute (cm)	Rf values
1	1.5	0.43
2	1.2	0.34
3	1.1	0.31
4	1.8	0.51

The distance travel by the solvent is 3.5cm

#### Acute Toxicity Study of Methanol Leaf Extract of *Raphanus raphanistrum*

The results of the oral acute toxicity (LD<sub>50</sub>) determination of Methanol Leaf Extract of *Raphanus raphanistrum* showed no death within 24 hours of extract administration in both phase 1 (10, 100 and 1000 mg/kg) and phase 2 (1600, 2900 and 5000 mg/kg) respectively (Table 4.3).

**Table 4.4: Acute Toxicity Study of Methanol Leaf Extract of *R. raphanistrum***

Experimental Phases	Dose (mg/kg)	Observation of death within 24 hours
Phase I	10	0/2
	100	0/2
	1000	0/2
Phase II	1600	0/2
	2900	0/2
	5000	0/2
LD <sub>50</sub> = > 5000 mg/kg		

There was no death of the rats in any of the phases during the acute toxicity testing of the experiment. This result indicated that the lethal dose (LD<sub>50</sub>) of *R. raphanistrum* is greater than 5000 mg/kg body weight of the experimental rats

#### Effects of Methanol Leaf Extract of *Raphanus raphanistrum* in Alloxan induced Diabetic Rats

There were no significant changes in the mean blood glucose level of the experimental animals before the induction of diabetes mellitus with alloxan monohydrate. The normal control groups maintained their blood glucose levels within the rat's normoglycemic of ≤ 100mg/dl throughout the period of the

study and the blood glucose level of the non-diabetic normal control group remained significantly lower compared with the diabetic control. Alloxan was able to induce hyperglycaemia after 48 hours of administration in all the test rats. Prior to the treatment no significant differences were observed in the blood glucose level of the diabetic control and the treated groups. The activity of the Methanol whole plant extract of *Raphanus raphanistrum* in

alloxan induced diabetic rats within 24 hours of administration reduced the blood glucose level except at the dose of 800 mg/kg in which an increase was observed after 8 and 24 hours respectively (Table 4.5). The activity of insulin after 1 hour of administration in diabetic rats significantly reduced hyperglycaemia when compared with the varying doses of the extract ( $p < 0.05$ ). However, the antidiabetic activity of the extract was not dose dependent at doses of 200, 400, 800 and 1600 mg/kg body weight within 24 hours of treatment. The plant extract at the dose of 200 mg/kg body weight significantly reduced blood glucose level among the diabetic rats when compared with the diabetic control group ( $p < 0.05$ ). The percentage reduction in blood glucose level was found to be higher among the lowest tested dose of 200 mg/kg after 1 hour (37%), 2 hours (28%), 8 hours (31%) and

24 hours (26%) post treatment. At 2 hours post treatment, the hypoglycaemic activity of insulin was observed to be significantly higher than the plant extract at 400 and 800 mg/kg ( $p < 0.05$ ). However, there were no statistical significant difference between the activity of the extract after 2 hours of administration and the diabetic control group ( $p > 0.05$ ) except at 200 mg/kg ( $p < 0.05$ ). The activity of the plant extract after 8 and 24 hours of administration was found to reduced hyperglycaemia which was not statistically significant when compared with insulin ( $p > 0.05$ ).

Conversely, the blood glucose levels in extract treated albino rats at the dose of 800 mg/kg after 8 and 24 hours of administration was found to increase. This shows that the plant extract at the dose of 800 mg/kg has lost its hypoglycaemic effect compared to others doses used (Table 4.5).



**Table 4.5: Effect of Methanol Leaf Extract of *Raphanus raphanistrum* on Blood Glucose Levels in Alloxan induced Diabetic Rats**

Treatment Group (mg/kg)	Changes in Blood Glucose levels within 24 hours of treatment (mg/dl)					
	A (mg/dl)	B (mg/dl)	1 hr	2 hr	8 hr	24 hr
NC (2ml/kg)	32.60±3.82	77.60±4.62	94.40±7.50 (22%) <sup>↑</sup>	85.40±8.53 (10%) <sup>↑</sup>	81.60±2.86 (5%) <sup>↑</sup>	91.20±1.24 (18%) <sup>↑</sup>
DC (2ml/kg)	54.40±6.99	283.80±46.15	307.40±53.74 (8%) <sup>↑</sup>	298.00±56.10 (5%) <sup>↑</sup>	351.20±80.71 (24%) <sup>↑</sup>	358.80±79.98 (26%) <sup>↑</sup>
IS 0.75 IU/kg	52.80±8.36	385.60±73.51	64.60±5.19 (83%)	175.40±12.32 (55%)	356.20±49.72 (8%)	450.40±50.11 (17%)
RR 200 mg/kg	44.40±1.40	370.60±70.56	234.20±75.38* <sup>β</sup> (37%)	267.40±77.58 <sup>β</sup> (28%)	254.20±88.19 (31%)	275.00±75.37 (26%)
RR 400 mg/kg	37.00±3.44	429.40±77.69	366.60±93.74* <sup>β</sup> (15%)	360.60±88.87* (16%)	363.80±92.95 (15%)	339.60±82.42 (21%)
RR 800 mg/kg	36.80±2.82	225.60±35.11	156.20±33.50* (31%)	177.40±42.02* (21%)	267.60±71.72 (19%) <sup>↑</sup>	294.00±62.70 (30%) <sup>↑</sup>
RR 1600 mg/kg	41.80±4.62	461.20±63.22	372.00±68.02* (19%)	371.60±64.05 (19%)	364.20±57.03 (21%)	349.80±69.02 (24%)
P-values	0.031	0.001	0.002	0.012	0.071	0.026

Values are expressed as mean ± SEM, (n = 5); one-way ANOVA. A (mg/dl) = blood glucose level before induction of diabetes with alloxan, B (mg/dl) = blood glucose level after 48 hours of alloxan administration (150mg/kg), NC = normal control, DC = diabetic control (untreated), IS = Insulin (Positive control), RR = *Raphanus raphanistrum*, \* = Compared with Positive control, <sup>β</sup> = Compared with Diabetic control, (p<0.05 is considered significant), ( ) = Percentage decrease in blood glucose level, ( <sup>↑</sup> ) = Percentage increase in blood glucose level

## Discussion

The phytochemical screening of Methanol leaf extract of *Raphanus raphanistrum* revealed the presence of carbohydrates, saponins, anthraquinones, glycosides, alkaloids, flavonoids, tannins. This is an agreement with the studies conducted by (Ramesh *et al.*, 2011).

Phytochemical screening of *Raphanus raphanistrum* (wild radish) using Methanol extract reveals a rich profile of bioactive compounds with potential therapeutic applications. The analysis indicates the presence of carbohydrates, tannins, glycosides, alkaloids, anthraquinones, and flavonoids, while saponins are notably absent (Farid *et al.*, 2022)

Carbohydrates, fundamental to cellular energy, suggest nutritional value in the plant. Tannins, known for their astringent and antimicrobial properties, may contribute to the plant's traditional use in treating infections. Glycosides, which often exhibit anti-inflammatory and cardioprotective effects, further enhance the medicinal potential of *R. raphanistrum* (Farid *et al.*, 2022).

Alkaloids, a diverse group of nitrogen-containing compounds, are pharmacologically significant for their analgesic, antimalarial, and anticancer activities. Their presence underscores the plant's potential in drug development. Anthraquinones, typically associated with laxative and antimicrobial effects, add to the therapeutic spectrum (Toro *et al.*, 2024).

Flavonoids, widely recognized for their antioxidant, anti-inflammatory, and

anticancer properties, are particularly valuable in combating oxidative stress and chronic diseases. Their presence aligns with findings from broader phytochemical studies that highlight *R. raphanistrum* as a source of potent antioxidants and enzyme inhibitors (Liu *et al.*, 2019).

Interestingly, saponins—commonly found in many medicinal plants for their cholesterol-lowering and immune-boosting effects—are absent in this Methanol extract. This absence may be due to the solvent's selectivity or the plant's inherent phytochemical composition.

Overall, the phytochemical profile of *Raphanus raphanistrum* supports its traditional medicinal use and suggests promising avenues for pharmaceutical and nutraceutical applications. Its diverse array of compounds could be harnessed for antimicrobial, antidiabetic, antioxidant and metabolic health interventions. Further studies, including compound isolation and bioactivity assays, would be valuable to fully explore its therapeutic potential.

The Rf (retention factor) values obtained from chromatographic analysis of *Raphanus raphanistrum* extracts offer important clues into the diversity and polarity of its phytochemical constituents. The Rf values recorded—ranging from 0.31 to 0.51—reflect the relative mobility of compounds in a chosen solvent system, which helps in characterizing the chemical profile of the plant. Extract 4, with the highest Rf value of 0.51, suggests the presence of less polar compounds. These compounds travel further

up the chromatographic medium due to weaker interactions with the polar stationary phase. This is typical of non-polar to moderately polar phytochemicals such as certain flavonoids, terpenoids, or hydrocarbons. Extracts with higher R<sub>f</sub> values may contain potential bioactive constituents with antioxidant, antimicrobial, or anti-inflammatory properties (Perez-Gutierrez and Perez, 2004).

Conversely, extract 3 has the lowest R<sub>f</sub> value of 0.31, indicating stronger interactions with the stationary phase and suggesting higher polarity. This could point to the presence of more polar constituents such as glycosides, tannins, or alkaloids—many of which have therapeutic relevance in traditional medicine (Farid *et al.*, 2022).

The variation in R<sub>f</sub> values among the four extracts underscores the chemical complexity of *R. raphanistrum*. It also implies a rich matrix of compounds with different polarities, paving the way for targeted isolation and identification using complementary chromatographic and spectroscopic methods (Iyda *et al.*, 2018).

These data not only validate the presence of bioactive compounds detected during phytochemical screening but also assist in the fingerprinting of extracts for quality control in pharmacognosy. Additionally, they guide future studies for identifying specific fractions with potential pharmacological benefits, enhancing the plant's value in drug discovery and natural product research (Ozen *et al.*, 2010).

Diabetes and its complications are one of the major health challenges globally and the optimum and rational use of the numerous plants adjuvant for the treatment of diabetes in traditional and natural indigenous systems of medicine in health care systems of any specific country is essential (WHO, 2008) but the efficacy and safety profile of these medicinal plants need to be evaluated. Thus, toxicity studies in animals are very important for the safety, development of new drugs and for the extension of the therapeutic potential of existing molecules. The toxicity profile of a medicinal plant in a subacute study; which is a 30-day study is well known for investigating any toxicity on long-term exposure to give valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at low dose on prolonged exposure that might be concealed and not observed in acute exposure or acutely nontoxic compounds.

### **Conclusion**

Phytochemical investigation of *Raphanus raphanistrum* shows the presence of carbohydrate, alkaloids, tannins, saponins, glycosides, flavonoids and anthraquinones.

The Methanolic Leaf extract of *Raphanus raphanistrum* demonstrated a moderate antidiabetic effect which was not dose dependent even though the activity is not comparable to that of the standard drug used (insulin). No any sign of toxicity or mortality was recorded during the oral acute toxicity and the lethal dose of ethanolic extract of *Raphanus raphanistrum* was found to be  $\geq 5000$  mg/kg body weight.

## References

- Al-Thwani, A.N.; Kais, K.G. and Rami, A.T. (2010). Effect of Ethaipathogenic *Escherichia coli* in vitro and in vivo. Iraqi J. Mark .Cons. Prot. 2 (4):166-181.
- Beevi SS, Narasu ML, Gowda BB(2010). Polyphenolics profile, antioxidant and radical scavenging activity of leaves and stem of *Raphanus sativus*. Plant Foods Hum Nutr. 2010;65:8-17.
- Beevi, S.S.; Mangamoori, L.N.; Dhand, V. and Ramakrishna, D.S. (2009). Isothiocyanate profile and selective antibacterial activity of root, stem, and leaf extracts derived from *Raphanus sativus* L. Foodborne Pathog Dis, 6: 129–136.
- Beevi,, L. N., Mangamoori, and B. B. Gowda (2012). Polyphenolics profile and antioxidant properties of *Raphanus sativus* L. Natural Product Research, 26(6), 2012, 557–563.
- Caceres, A. (1987). Screening on antimicrobial activity of plant popular in Guatemala for the treatment of dermato mucosal diseases. J. Ethnopharm, 20: 223-237..
- Farid, M., Ansar, B. A., Ushie, O. A., Ahmad, K. B., Abdullahi J. A., and Nasir, A. M. (2022). Phytochemical screening and antioxidant activity of medicinal plants: Absence of saponins in selected species. \*Pakistani Journal of Botany\*, 54(3), 885–892.
- Gutiérrez, R. M., Mitchell, S., and Solis, R. V. (2007). Traditional uses, chemical constituents, and biological activities of \*Phyllanthus niruri\*. \*Journal of Ethnopharmacology\*, 109(3), 490–500.
- Harborne, J.B. and Baxter, H. (1993). Phytochemical Dictionary. A hand book of Bioactive compound from plants. Taylor and Francis, London.
- Ihedioha, T. E., Omoja, V. U. and Asuzu, I. U. (2014). Effects of Methanolic stem bark extract of *Cassia sieberiana* DC on fasting blood glucose and serum lipid profile of alloxan induced diabetic rats. *Animal Research International*; **11**(1): 1871 – 1880
- Iyda, O. E., Yi, Y., Zheng, J., and Hamid A. M.. (2018). A complementary liquid chromatography–ion mobility–mass spectrometry method for structural isomer separation. \*Frontiers in Molecular Biosciences\*, 5, 95.
- Jain, D. K. and Arya, R. K. (2011). Anomalies in alloxan-induced diabetic model: It is better to standardize it first. *Indian J Pharmacol.*; 43:91.
- Jayakar, B. and Suresh, B. (2003). Antihyperglycemic and hypoglycemic effect of *Aporosa lindleyana* in alloxan induced diabeic rats. *Journal of Ethnopharmacology*; **84**: 247-249.
- Johansen, J.S, Harris AK, Rychly DJ, Ergul A.(2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical

- practice. Cardiovasc Diabetol.;4(1):1–11.
- Jones L J L, Thorpe J P and Wallis G P. (1982). Genetic divergence in four species of the genus *Raphanus*: Implications for the ancestry of the domestic radish *R. sativus*. J. Linnean Society. 18(1): 35-48.
- Karadag A, Ozcelik B, Saner S. (2009). Review of methods to determine antioxidant capacities. Food Anal Methods. 2009;2(1):41–60.
- Kim, W.K.; Kim, J.I.; Jeong, D.H.; Chun, Y.H.; Kim, S.H.; Cho, K.J. and Chang, M.J. (2011). Radish (*Raphanus sativus* L. leaf) Methanol extract inhibits protein and mRNA expression of ErbB2 and ErbB3 in MDA-MB-231 human breast cancer cells. Nutr Res Pract, 5(4): 288-293.
- Krishna, D., Sarbatly, R., and Nithyanandam, R. (2012). A review of the antioxidant potential of medicinal plant species. \*Food and Bioproducts Processing\*, 90(2), 217–233.
- Liu, H.; Kang, Y.; Zhao, X.; Liu, Y.; Zhang, X.; Zhang, S. (2019). Effects of elicitation on bioactive compounds and biological activities of sprouts. J. Funct. Foods 2019,53, 136–145.
- Lobo V, Patil A, Phatak A, Chandra N. (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 4(8):118.
- Lugasi A, Blazovics A, Hagymasi K, Kocsis I, Kery A. (2005). Antioxidant effect of squeezed juice from black radish (*Raphanus sativus* L. var niger) in alimentary hyperlipidaemia in rats. Phytother Res. 2005;19(7):587-91. Food Sci. 2006;71(2):144-8.
- Lugasi, A.; Blazovics, A.; Hagymasi, K.; Kocsis, I. and Kery, A. (2005). Antioxidant effect of squeezed juice from black radish (*Raphanus sativus* L. var niger) in alimentary hyperlipidaemia in rats. Phytother Res, 19(7): 587–591.
- Mohammed, A. (2013). Effect of drying and extraction methods on antioxidant activity of selected vegetables. *Journal of Food Processing and Technology*, 4(6), 1000226.
- Nakamura, Y., Yoshimoto, M., Murata, Y., Shimoishi, Y., Asai, Y., and Park, E. Y. (2008). Comparison of antioxidative compounds and reactive oxygen species scavenging activity in colored radishes. \*Journal of Agricultural and Food Chemistry\*, 56(22), 10502–10508.
- Özen, T. (2010). Antioxidant activity of wild edible plants in the Black Sea Region of Turkey. *Grasas y Aceites*, 61, 86–94.
- Papi, A.; Orlandi, M.; Bartolini, G.; Barillari, J.; Iori, R.; Paolini, M.; Ferroni, F.; Grazia, F.M.; Pedulli, G.F. and Valgimigli, L. (2008). Cytotoxic and antioxidant activity of 4-methylthio-3-butenyl isothiocyanate from *Raphanus sativus* L. (Kaiware Daikon) sprouts. *J Agri Food Chem.*, 56: 875–883.

- Paredes, S.D. (1984) Etnobotánica Mexicana: Plantas popularmente empleadas en el Estado de Michocán en el tratamiento de enfermedades hepáticas y vesiculares. Tesis Lic. México
- Perez-Gutierrez, R.M. and Perez, R.L. (2004). *Raphanus sativus* L. Radish, Their chemistry and biology. The Scientific World J., 4: 811-837.
- Pritô, P., Pineda, M., and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337–341.
- Ramesh CK, Raghu KL, Jamuna KS, Joyce GS, Mala RS, Avin BR(2011), Comparative evaluation of antioxidant property in Methanol extracts of some common vegetables of India. *Annals of Biological Research*: 2: 86-94
- Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202–1205.
- Shukla, S.; Chatterji, S.; Yadav, D.K. and Watal, G. (2011). Antimicrobial efficacy of *Raphanus* constituents of some Nigerian medicinal plants. *Africa. J. Biotechnol*, 4: 685–688.
- Sofowora A. (2008) Medicinal plants and traditional medicine in Africa. 2nd edition, Spectrum Book Limited, Ibadan, Nigeria, 134-156.
- Toro, G., Khan, R., Ahmed, S. and Husseini, R.S (2024). Evaluation of antimicrobial activity of essential oils against selected bacterial strains. *\*Sarhad Journal of Agriculture\**, 40(S1), 110–121.
- Trease, G. E., and Evans, W. C. (2002). A text book of Pharmacognosy *Pharmacognosy\** (15th ed.). Bailliere, Tindals London, 1989 176-180: W.B. Saunders.
- Weilan, W.; Jin, Z.; Zhongda, L. and Menga, L. (1987). Hypotensive constituents of laifuzi. *Zhongcayao*, 18: 101-103.
- World Health Organization (WHO) (2008). Africa Traditional Medicine. *Medicinal Journal of Australia*, 30:224-226.
- Yamasaki, M.; Omi, Y.; Fujii, N.; Ozaki, A.; Nakama, A.; Sakakibara, Y.; Suiko, M. and Nishiyama, K. (2009). Mustard oil in “Shiboriaikon” a variety of Japanese radish, selectively inhibits the proliferation of H-ras-transformed 3Y1 cells. *Biosci Biotechnol Biochem*, 73(10): 2217–2221.
- Yong, J.K.; Kug, C.Y.; Min, K.H. and Kyu, C.B. (2000). Radish extract stimulates motility of the intestine via the muscarinic receptors. *J. Pharmacol.*, 52 : 1031-1036.