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EVALUATION OF PHYTOCHEMICAL, ANTIMICROBIAL, *IN-VITRO* ANTIOXIDANT & ANTI-INFLAMMATORY STUDY OF METHANOLIC EXTRACT OF *RAPHANUS SATIVUS* FRUIT

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Keywords:

Raphanus sativus , Anti-microbial, Anti-inflammatory, Antioxidant, Radish root

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ABSTRACT: *Raphanus sativus* L, belongs to the family Brassicaceae. Phytochemical analysis of its methanolic root extract revealed the presence of alkaloids, flavonoids, glycosides, tannin, phenolic compounds, triterpenoids and steroids. The antimicrobial activities and *in-vitro* Pharmacological activities like Antioxidant Activity and Anti-inflammatory activity of the root extract was assessed. Production of reactive oxygen species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. In this study the radical scavenging ability different extracts of *Raphanus sativus* L. was undertaken. Results revealed that the root of *Raphanus sativus* L. has potent antioxidant ability. A good correlation was found to exist between concentration of extract and % inhibition of protein denaturation and free radicle damage. From different literature reports, root extract was found to be more potent antioxidant than the other parts of the plant. The extract had shown significant anti-bacterial activity against bacterium like *Lactobacillus*, *Streptococcus aureus*, *Escherichia coli* against standard Tetracycline by the determination of Minimum Inhibitory Concentration and Zone of Inhibition. The protein denaturation study reveals potent anti-inflammatory activity by significant reduction in turbidity. Thus, radish root extract has proved to be considered as an effective medicinal herb for both of its Anti-microbial and Pharmacological properties.

INTRODUCTION: *Raphanus sativus* L. is a cruciferous vegetable which belongs to the family of Brassicaceae, is commonly known as radish or daikon and widely consumed all over the world as a vegetable in human diets. The most popular part for consumption is the taproot, although the entire plant is edible and the aerial part is also used as leaf vegetable.

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Different parts of radish, such as roots, seeds, flowers, sprouts, and leaves, have been known to show various medicinal properties [1]. Throughout the research radish has been reported to possess a wide range of pharmacological activities, such as hepatoprotective, cardioprotective effect, anti-diabetic activity [2].

Meanwhile, pharmacological studies have also shown that radish roots possess a wide range of biological activities, such as healing ulcers in patients suffering from peptic ulcer disease, and antihypertensive effect in spontaneously hypertensive rats. Besides this, reports has shown that the ethanol extracts from radish root may be a

clinically useful anti-inflammatory agent by suppressing the COX-2 activity. Several studies demonstrated that radish root and radish root extract have a preventative effect on excessive reactive oxygen species (ROS) generation and thus it acts as an effective anti-oxidant [3]. Radish's biological activities are due to the presence of several classes of phytoconstituents such as alkaloids, phenols, and flavonoid compounds. Flavonoid compounds known for their nutraceutical and pharmacological properties are among the most important of these phytochemicals in *R. sativus*. Rutin which is a flavonoid compound [4], represents flavonoids that naturally and widely occurs in several fruits as well as vegetables. It contains powerful antioxidant and antimicrobial properties and several nutritional and beneficial effects. *Raphanus sativus* roots contain sulforaphene compound, responsible for its strong antifungal activity [5, 8]. Radish sprouts has shown to be effective for inhibition of growth of different microbes like *Escherichia.coli, Pseudomonus aurignosa, Bacillus subtilis* [6], *Staphylococcus aureus, Enterococcus faecalis* [7, 9] *etc*. This study is aimed to evaluate the phytochemical analysis of radish root and analyze the antimicrobial broad-spectrum, antioxidant, anti-inflammatory property thus a cost- effective easily available vegetable can be an option of treatment for various life threatening diseases.

MATERIALS & METHODS:

Collection and Authentication of Plant: The roots of *Raphanus Sativus* (around 5 kg) were collected from local market of Panskura during the month of January 2022 and the plant was identified by the help of regional nursery and finally authenticated by Botanical Garden of West Bengal (Authentication Number – JIS/S-01).

List of Chemicals Required:
For Extraction: Methanol

For Antioxidant Study: Hydrogen peroxide, Ascorbic Acid, Potassium Dihydrogen Phosphate, Sodium Hydroxide.

For Anti-inflammatory Study: Bovine Serum Albumin, Phosphate Buffer, Diclofenac.

For Anti- microbial Study- Nutrient Agar

Preparation of Plant material for Extraction: Fresh Radish root, after collection was sun dried for 7 days. Plant material was then grinded in a mixer-grinder. Then they were milled to a coarse powder transferred through sieve and stored in an air tight container.

Extraction Procedure: About 180 g of coarse powder was taken into a conical flask and 500 ml of Methanol. Then it was wrapped in aluminum Foil and allowed to macerate for about 7 days with periodical shakings. After cold maceration, the extract was filtered and the excess solvent was reduced using a rotary evaporator. The semisolid extract was refrigerated at 4°C for further analysis [10].

Phytochemical Analysis of methanolic Extract of Radish Roots: The phytochemical studies like test for alkaloids, glycosides, steroid, saponin, polysaccharides were done to identify the presence of various secondary metabolites which are the cause of numerous health benefits of the plant [11].

TABLE 1: VARIOUS PHYTOCHEMICAL TESTS USING *R. SATIVUS* FRUIT EXTRACT

Sl. no.	Test	Reagent	Observation
1	Modified Brontrager's Test	$Fecl_3$+Hcl+$Chcl_3$	Red Colour Layer
2	Steroid Test	Con^c H_2so_4	Lower Layer Red Colour
3	Alkaline Reagent Test (Flavonoids Test)	NaOH Solution+ Dil. HCl	Colourless
4	Zinc Hydrochloride Test (Flavonoids Test)	Zn Dust + Conc. HCl	Light Red Colour
5	Ferric Chloride Test (Tannin Test)	$Fecl_3$	No Colour Change
6	Baljet's Test (Cardiac Glycoside)	Picric Acid Or Sodium Picrate	Orange Colour
7	Mayer's Reagent (Alkaloids Test)	Mayer's Reagent (Potassium Mercuric Iodide Solution)	Precipitation Formed
8	Dragendroff Test	Potassium bismuth iodide	No Colour Change
9	Keller-Killiani Test (Cardiac Glycoside)	Pb(C2h3o2)2 + Chcl3 + Glacial Ch3cooh + Fecl3 + H2so4	Redish Brown Layer Not Formed
10	Iodine Test (Poly Saccaride)	Iodine Solution + HCl	No Colour Change
11	Froth Formation Test (Saponin Glycoside)	Water	Foam Formed
12	Tri-Terpinoied Test (Salkowski)	Chcl3 + Conc. H2so4	Yellow Colour Upper Layer

Antioxidant Activity:

H_2O_2 Radical Scavenging Activity:

Preparation of Phosphate Buffer (pH 7.4): 3.4g of KH_2PO_4 (Potassium dihydrogen phosphate) + 0.75g of NaOH (sodium hydroxide) were dissolved in 500 ml of Distilled Water.

Preparation of 40mM of Hydrogen Peroxide: 4.420 mL of H2O2 (30%) was dissolved in 50mL of phosphate buffer (pH7.4) to final 40 mM concentration. Fresh H202 was prepared before the experiment, and put in a dark glass amber volumetric flask. Fresh solution was prepared each time before the experiment.

Preparation of Standard solution (Ascorbic Acid): 10mg of ascorbic acid was dissolved in 10mL of phosphate buffer (pH7.4) and this solution was used as a standard.

Procedure of H_2O_2 Radical Scavenging Activity: The antioxidant activity of the extract was evaluated in vitro by hydrogen peroxide radical scavenging method. 1 ml of the dilutions of the extract (40, 60, 80 and 100 µg/ml) were added to the tube containing 0.6 ml of 40 mM hydrogen peroxide solution. The resulting solutions were incubated for 10 minutes at 370° C and the absorbance of the solution was measured at 230 nm against phosphate buffer as a control and Ascorbic acid as standard [12]. The percentage hydrogen peroxide radical scavenging activity was determined using the formula:

% Radical Scavenging Activity = (Absorbance of test / Absorbance of control - 1) ×100

Anti-Inflammatory Activity:

Preparation of Phosphate Buffer (pH 7.4): 3.4g of KH_2PO_4 (Potassium dihydrogen phosphate) + 0.75 g of NaOH (sodium hydroxide) were dissolved in 500 ml of Distilled Water.

Procedure: *In-vitro* Albumin denaturation method 13 was used to evaluate the anti-inflammatory activity of the extract. 1 ml of the extract dilution (20, 40, 60, 80 and 100 µg/ml) was added to 1 ml of 1% bovine albumin solution prepared in phosphate buffer (pH 7.4) and was incubated at 37°C for 15 minutes. Denaturation was induced by placing the test tubes in a water bath at 600°C for 10 minutes. After cooling, the turbidity produced by denaturation was measured using visible spectrophotometer at 660 nm against phosphate buffer. Diclofenac was used as a standard anti-inflammatory agent [12].

Phosphate Buffer solution was used as control. All the experiments were performed in triplicates. The percentage inhibition of denaturation was measured using the formula:

% Inhibition = (Absorbance of test / Absorbance of control - 1) × 100

Anti-Microbial Study:

Determination of Minimum Inhibitory Concentration: At first all the apparatus required for the antimicrobial study should be autoclaved properly, Water used in the study is also sterilized. Nutrient broth is prepared. 8 conc. of the Sample Extract is prepared using 1% DMSO (Dimethyl sulfoxide). Then each of them is dissolved in 3ml of broth in Test Tubes.

One loop full of each bacterium, *Lactobacillus*, *Streptococcus aureus* and Escherichia coli is given to each of the test tubes. Then the test tubes are incubated for 24 hrs at 37°C in BOD incubator. After 1 day Turbidity is checked for each test tube. The dilutions in which the solutions appear to be clear is regarded as the Inhibitory Concentrations. The Minimum concentration of sample at which the antimicrobial activity is observed i.e the solution appears to be clear is taken as Minimum Inhibitory Concentration of the sample for showing Anti-microbial activity. All the experimental procedure should be conducted inside an Aseptic Area.

Determination of Zone of Inhibition: Agar media is prepared by dissolving 28g Nutrient Dust Agar in 1000ml of water. All the apparatus and also nutrient agar is autoclaved properly. Then the liquid agar is made to solidify and kept in incubator for 24 hrs. After 24 hrs the agar plates are checked for any contamination due to operational errors and contaminated plates are discarded.

Divide the plates in 4 sections using marker .1ml Broth of each bacterium is poured in the agar plate and incubate for 1 hour. Prepare the Test Sample Solution and tetracycline solution according to concentration. Then Small Discs of Whatman Filter

paper dipped in those solutions. After 1 hour, the filter paper discs are placed in the designated places inside the Agar plates by using Forceps. Then the Agar plates are transferred to BOD Incubator and kept for 24 hours at 37°C. All the experimental procedures should be conducted inside Laminar Air Flow by maintain proper aseptic conditions. Then the diameter of the zone of Inhibition is checked after 1 day by using a scale [13].

RESULT:

Phytochemical Analysis of Radish Roots:

TABLE 2: PRESENCE OF PHYTOCHEMICAL IN R. SATIVUS FRUIT

Phytochemicals Study	Methanolic Extract of *Raphanus Sativus* Roots
Alkaloids	(+)
Flavonoids	(+)
Tannins	(+)
Saponins	(+)
Terpenoids	(+)
Glycoside	(+)

(+): Presence of Constituents (-): Absence of Constituents.

Study of Minimum Inhibitory Concentration (MIC):

TABLE 3: STUDY OF MIC USING *R. SATIVUS* FRUIT

Concentrations(g/ml)	0.025	0.050	0.075	0.100	0.125	0.150	0.175	0.200
Lactobacillus(ATCC-8014)	+	+	+	+	+	+	+	**(-)**
Streptococcus aureus (ATCC-9144)	+	+	+	+	**(-)**	+	+	+
Escherichia coli(ATCC- 10231)	+	+	+	+	+	+	+	**(-)**

(+): Bacterial Growth Visible (-): Bacterial Growth Inhibited.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION

Microorganisms	Minimum Inhibitory Concentration
Lactobacillus	0.200g/ml
Streptococcus Aureus	0.125g/ml
Escherichia coli	0.200g/ml

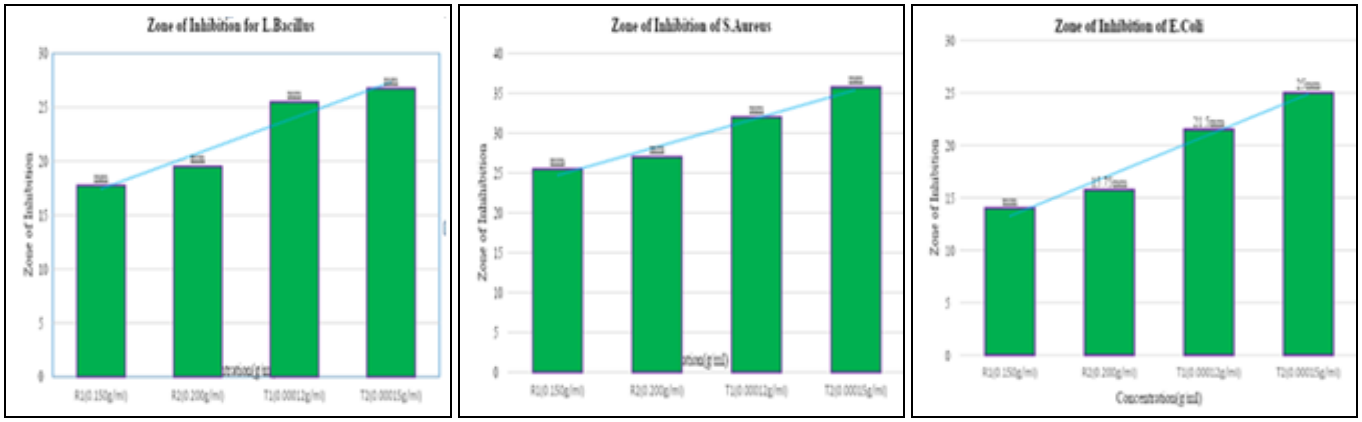

FIG 1: COMPARISON OF ZONE OF INHIBITION BETWEEN *R. SATIVUS* EXTRACT AND TETRACYCLINE IN THREE TYPES OF BACTERIA

FIG. 2: ZONE OF INHIBITION

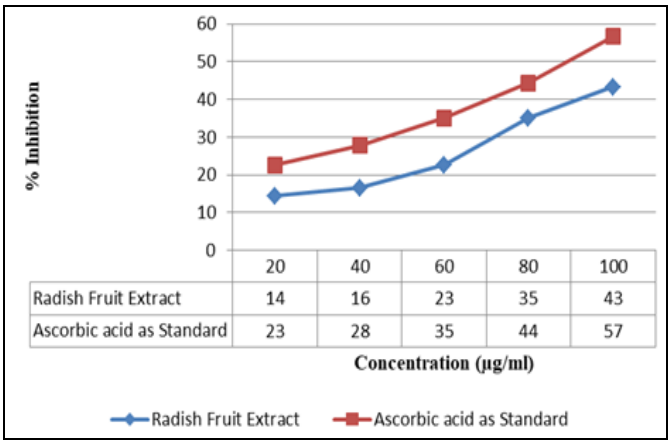

FIG. 3: *IN-VITRO* ANTIOXIDANT STUDY AGAINST STANDARD ASCORBIC ACID

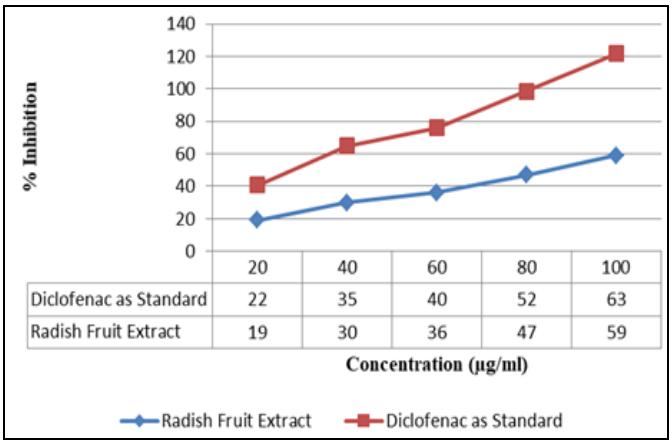

FIG. 4: *IN-VITRO* ANTI-INFLAMMATORY STUDY AGAINST DICLOFENAC

DISCUSSION AND CONCLUSION: The methanolic extract of radish fruit/root has proved the presence of various important phytochemical substances which shows numerous health benefits. The study shows that the radish roots consist of glycoside, alkaloids, flavonoids, terpinoids, saponin and tannin **Table 1**. According to the article written by Rehab A. Hussein and Amira A. El-Anssary on "Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants" Flavonoids which are the phenolics promised to have antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, antianaphylactic, antimutagenic, choleretic and bronchodilatory actions. It's also helpful in preventing insulin degradation thus can be helpful as anti-diabetic.

The tannins are also polyphenolic compounds which are mainly helpful to precipitate protein. This principle is being utilized in leather industry to precipitate the protein in raw animal. Saponin has proved to have various pharmacological effects like antitumor, piscicidal, molluscicidal, spermicidal, sedative, expectorant and analgesic properties.

Alkaloids demonstrate a diverse array of pharmacological effects including analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation as well as antineoplastic, hypertensive and hypotensive benefits [14]. In case of anti-microbial study. The anti-microbial study was done using three microorganisms, two gram positive (*Lactobacillus* and *Streptococcus aureus)* and one gram negative (*E. coli*). In this study tetracycline which is a broad spectrum antibiotic has been taken as a reference or standard anti-microbial substance. At initial level MIC (Minimum Inhibitory Concentration) has been determined for each of the bacteria using various concentration of the extract (0.025 g/ml, 0.050 g/ml, 0.100 g/ml, 0.125 g/ml, 0.150 g/ml, 0.175 g/ml, 0. 200 g/ml). This study followed the turbidimetric method where the turbidity after one day or 24 hours of incubation has been checked of each concentration on UV-Vis spectrophotometer on 530 nm wavelength. The concentration where we couldn't see turbidity at all or the solution transparency was exactly same like that was 24 hours before that concentration can actually considered as MIC. For the confirmation the absorbance is checked and compares that with the next concentrations.

Radish roots extract at concentration 0.200 g/ml could inhibit the *Lactobacillus* and *E. coli* but at concentration 0.125g/ml it has inhibited *Steptococcus aureus* **Table 4**. The zone of inhibition which demonstrate the potency of a compound to inhibit the growth of a specific microorganism, comparing that with a standard anti-microbial substance.

Radish root has showed dose dependent increase in response in terms of preventing microbial growth while two concentrations of tetracycline is used as a reference standard. The outcome in MIC and Zone of Inhibition proves the strong anti-bacterial effects of radish root which is a vegetable of regular consumption may protect the human lives from bacterial infection like Strep throat, scarlet fever, blood infection, pneumonia, stomach cramps, bloody diarrhea, vomiting *etc* [15, 16]. *In-vitro* anti-oxidant study was performed using hydrogen

peroxide (H_2O_2) as a scavenging agent and Ascorbic acid as standard antioxidant which will prevent the free radicle damage. The study showed the dose dependent increase of response in terms of preventing free radicle damage of the cell. Here bovine serum albumin was taken as sample and various concentrations like 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml of extract and reference standard ascorbic acid was taken against H_2O_2. The result has been achieved which was quite commendable as the free radicle scavenging activity of the extract was quite near to the activity of ascorbic acid **Fig. 3**. Inflammation is a symptom which is very common to most of the type of degenerative disorders thus having an anti-inflammatory activity in a drug can be a promising effect. Here in this study diclofenac which is a standard drug comes under NSAID (non-steroidal anti-inflammatory drug). Inflammation has been induced by over-heating the sample. Various concentrations of extract (20µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) have been used to prevent the inflammation induced by protein denaturation. Same concentration of reference standard drug diclofenac has been used to do a comparative analysis. Highest concentration (100µg/ml) of diclofenac inhibited 63% of protein denaturation induced inflammation where radish fruit extract inhibited 59% which is truly commendable in terms of potency **Fig. 4.**

Radish Fruit (*Raphanus sativus*) which is easily available and pocket friendly vegetable is showing a multi-directional benefits such as broad spectrum anti-microbial, anti-oxidant and anti-inflammatory effects. Having all these effects in one indicates multi-dimensional activity to prevent or treat many degenerative disorders which are caused by various micro-organisms, free radicle damage or due to protein denaturation induced inflammation of cell. Further *in-vivo* study will help to clarify the above mentioned effect in terms of further research prospective.

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