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STRATEGIC REVIEW ON RAPHANUS SATIVUS LEAVES

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ABSTRACT

Recently much attention has been given to the anti oxidant capacity of natural products especially those present in the human diet and their by products such as vegetables. The cruciferous vegetables are a good source of natural anti oxidants. Radish is an important vegetable of cruciferous family which is used world wide. Hence in the present study the leaf of radish has been mainly focused and this review systematically addresses and summarises the various phytochemicals and pharmacological action of radish leaves.

KEYWORDS: *Raphanus sativus*, Polyphenols, Terpenes, Flavonoids, Micro nutrients.

INTRODUCTION

Radish is an edible root vegetable of the family Brassicaceae. Radishes are grown and consumed throughout the world, being mostly eaten raw as a crunchy salad vegetable with a pungent and bitter flavour.

Radish owe their sharp flavour to the various chemical compounds produced by the plants, including glucoisinolate, myrosine and isothiocyanates.

Sub species of radish

Daikon - *Raphanus raphanistrum sub sp. Sativus* (syn. *Raphanus sativus var.longipinnatus)*

Green radish – *Raphanus sativus var. caudatus*

Black radish – *Raphanus sativus var. niger*

Oilseed radish – *Raphanus sativus var. oleiformis*

Wild radish – *Raphanus sativus var. raphanistroides*

Red radish – *Raphanus raphanistrum sub* sp. *sativus*

Raphanus sativus L, commonly known as radish is a cruciferous plant that is consumed worldwide in various forms. However, the radish leaves are discarded after harvest. In addition, many studies are done on radish roots for its beneficial effects. While, research on pharmacological activities of radish leaves are limited.

Radishes are annual or biennial brassicaceous crops grown for their swollen tap roots which can be globular, tapering, or cylindrical. The radish is a diploid species, and has 18 chromosomes (2n=18). It is estimated that the radish genome contains between 526 and 574 Mb.[1]

Nutrients and phytochemicals were reported mainly in roots and leaves. The largest categories were Flavonoids, Non-flavonoids, Polyphenols, Terpenes and derivatives, Fat and Fatty related compounds and Glucosinolates and breakdown products.[2]

Taxonomical classification[3]

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Sub phylum: Angiospermae

Class: Dicotyledonae

Order: Capparidales

Family: Brassicaceae

Genus: Raphanus

Species: *Raphanus sativus*

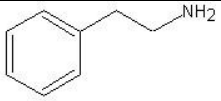
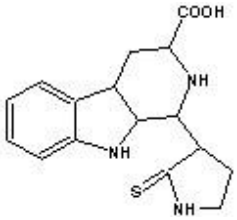
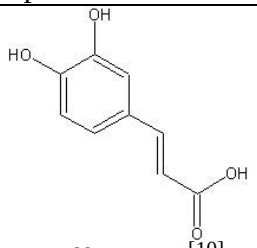
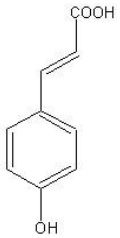
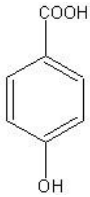
Leaves description

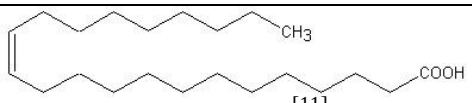
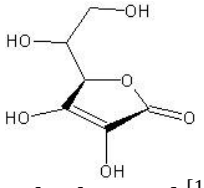
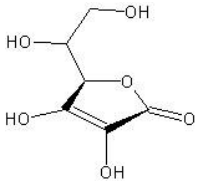
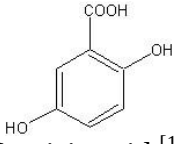
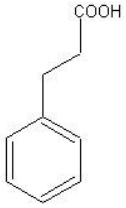
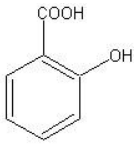
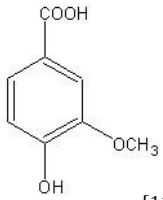
Leaves are alternate, glabrous to sparingly hispid; lower leaves in a radical rosette, petioles 3-5.5 cm long, leaf-blades oblong, oblong-ovate to lyrate-pinnatifid, 3-5-jugate with a round or ovate terminal lobe, 5-30 cm long; higher leaves much smaller, shortly petioled, lanceolate-spathulate, subdentate.[3]

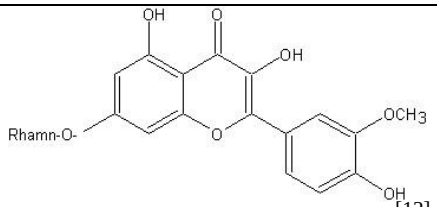
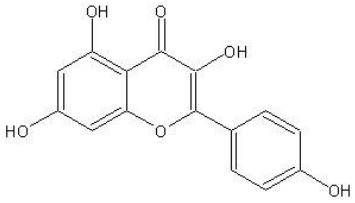
Leaf is a thin, dorsiventral flattened organ of a vascular plant having appendage with the stem and responsible for photosynthesis. Leaves and stem together form shoot, whereas the entire shoot form the foliage. The immature leaves of foliage are soft but have high metabolic rate and possess chlorophyll, a green colored pigment an important quality attribute. The leaves of

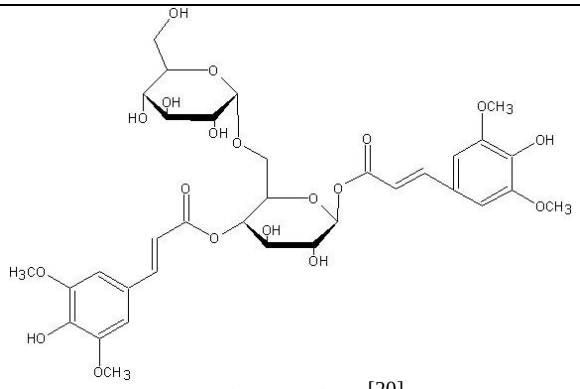
radish are good source of protein and found to have biological value of 76.6 with the digestibility co-efficient of 73.5% may be due to the presence of nitrogenous fraction of present twenty two amino acids.[4]

Active constituents[5]

Alkoloids and Nitrogen compounds	 Phenethlamine[6]  1-(2-pyrrolidinethiol-3-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxilic acid[6]
Enzymes.	Anionic peroxidase; A1, A2, A3, A4[7] Arabino galactan protein[8] Catalase glulathione reductase[9] ß-fructosidase(ß-F) Superoxide dimutase.
Organic acid.	 Caffeic acid[10]  p-coumaric acid[10]  p-Hydroxy benzoic acid[10]

	 Erucic acid.[11]  Erythrobic acid.[11]  Ferulic acid.[11]  Gentisic acid.[11]  Hydrocinnamic acid.[11]  Salicylic acid.[11]  Vanilic acid.[11] Linoleic acid Linolenic acid Malic acid, Melanoic acid Oxalic acid

	Palmitic acid.
Phenolic Compounds.	 Isorhamnetin-7-0-rhamnoside.[12]  Kaempferol. Gallic acid Procatechuic acid p-hydroxy benzoic acid Chlorogeicacid Sinapic acid 2-methoxy-4-methylphenol.
Polysaccharide.	Pectic substance[13] Rhamnose[13] Glucose[13] Xylose[13]
Proteins.	Arabinogalactan Proteins(AGPs) L-arabino-D-galactan & Arabino-3-6-galactan.[14] S-glycoproteins Polypeptides RCA1, RCA2, RCA3.[15] Ferredoxin isoprotein.
Sulfur.	$H_2C=CHCH_2NCS$ Allyl-isothiocyanate.[16] CH2=CHCH2CH2C(SGLc)=NOSO3H Sinigrin.[17] (S)-sulfonium form of S-adenosylmethionine.[18] 6-methyl-sulfinyl hexyl-isothiocinate. 5-vinyl-2-oxazolidinethione.[19]
Other.	Caratenoids

	 Raphanusol A.[20] Raphanusol B.[20]

Uses[21]

- Increase immunity and reduce fatigue
- For treatment of piles
- Treatment of jaundice
- Prevents Diabetes
- It is a detoxifying agent and also helps in digestive process
- Relieves rheumatism
- Anti- Scorbutic
- Radish leaves are naturally diuretic
- Antioxidant
- Anti inflammatory

Pharmacological activities that have been proved on radish leaves were

1. Anticancer activity

Seung-u-son, et,al in this study they have reported peptic polysaccharides were isolated from dried radish leaves by hot water extraction and subsequent precipitation was done using 80% ethanol to investigate their effects on the innate & adaptive immune system.

HPLC method was applied for determining the sugar composition & molecular weight of polysaccharide.

Effect of polysaccharides on viability and cytokines production of peritoneal macrophages in vitro, NK cells activity, inhibitory effect on lung metastasis and oral administration of polysaccharides on anti metastatic effect were studied.

Hence polysaccharide was confirmed to have highly potent anti tumor metastasis effect via immunostimulating activities which makes it a good candidate for developing functional foods and pharmaceuticals.[22]

Woo Kyoung kim, et.al., reported the effect of ethanolic extract of aerial parts of *Raphanus sativus L*(ERL) on breast cancer cell proliferation and gene expression associated with cell proliferation and apoptosis in MDA-MB-231 human breast cancer cells.

In breast cancer, the epidermal growth factor receptor (EGFR) is an important oncogene. It is composed of family members including ErbB1, ErbB2, ErbB3 and Erb4. ErbB2 gene amplification has been associated with the development of breast cancer in animal models.

MTT assay was performed to evaluate the inhibitory effect of ERL on cancer cell proliferation.

ERL reduces protein and mRNA expression of ErbB2 in MDA-MB-231 cells and also reduces protein & mRNA expression of Akt MDA-MB-231 cells.

ERL increases protein & MRNA expression of Bax and Bcl2 in MDA-MB-231 cells.

This study confirms the ERL is capable of inducing apoptosis in MDA-MB-231 human breast cancer cells.[23]

Omar M Noman, et.al., have studied that 70% ethanol extract followed by maceration procedure of *Raphanus sativus L*. Leaves effect on the proliferation and viability of two breast (MCF-7 & MDA-MB-231), liver (Hep G2), and lung (A549) cancer cells were evaluated using MTT assay.

From the above MTT assay results revealed that *Raphanus sativus L*. exhibited antiproliferative activity against all the tested cancer cells. The viability of all the treated cells decreased in dose dependent manner and *Raphanus sativus L*. extract Posses highest antiproliferative efficiency against all cell lines with IC50 values of 217-453 microgram per millilitre.

From this study it was observed that *Raphanus sativus L*. extract shows inhibitory effects on cancer cell proliferation.[24]

A.Umamaheswari et.al.,the current study includes the green synthesis of ZnO nanoparticles (ZnO NPs) from the leaves of *Raphanus sativus var. Longipinnatus* and interpretation of its anticancer activity.

ZnO NPs were synthesised using ethanolic extract of radish with zinc acetate. The prepared ZnO NPs were investigated by UV-vis, FTIR, particle size analysis, SEM, XRD analysis. Then the bio synthesised ZnO NPs was analysed for cytotoxic activity using in vitro technique for A549 cell lines by MTT assay.

It is observed that cell viability percentage was sharply reduced and IC50 was found to be 40 microgram per millilitre. Higher surface area to volume ratio of these small particles may be responsible for enhanced cytotoxic effect.

Flavanoids and phenol were found as phytoconstituents which played a role as capping or stabilising agent in the metal NPs synthesis.

This biosynthesized ZnO NPs had a better anticancer activity proving that cancer drugs can be prepared by this constituted ecofriendly technique.[25]

Rama Koyyati, et.al., studied the aqueous leaf extract of *Raphanus sativus var. Longipinnatus* was treated with cobalt acetate solution to prepare the cobalt nanoparticles(CoNPs). These prepared CoNPs characterised by using UV-vis, FTIR, SEM-EDX analysis. In vitro study of cytotoxicity effect on HeLa cell line was assessed by MTT assay.

Percentage cytotoxicity was calculated and used for finding IC50 value of the concentration required for 50% cell death by synthesized CoNPs.

This Study reported that biologically synthesised CoNPs could be of immense use in medicine for their cytotoxic properties and in vitro screening of CoNPs showed potential cytotoxic activity against the HeLa cell lines.[26]

2. Antimicrobial activity

Rama Koyyati, et. al., synthesised the cobalt nanoparticles using the aqueous extract of *Raphanus sativus var. Longipinnatus* leaf. These synthesised nanoparticles are characterized using various techniques such as UV- visible spectroscopy, FTIR spectroscopy, SEM – EDX analysis.

Antibacterial activity of synthesised CoNPs against Gram negative organisms like *Pseudomonas putida* and *Klebsiella pneumonia* were studied using Disc diffusion method then the zone of inhibition was measured 10mm and 8.5mm respectively and compared the results with standard antibiotic (Ampicillin), leaf extract and 10mM cobalt solution.

The results indicate that cobalt nanoparticles synthesised from *Raphanus sativus var. Longipinnatus* leaf extract showed effective antibacterial activity against gram negative bacteria.[26]

Sangeetha K, et.al., reported that hydroalcoholic (ethanol 95% w/w) extract of *Raphanus sativus* was tested for anti microbial activity against *Bacillus subtitles, Pseudomonas sp, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus* bacterial pathogens. The antibiotic susceptibility pattern or anti microbial activity of the pathogens was determined by well diffusion method on Nutrient Agar plates.

The hydroalcoholic extract exhibit strong anti microbial activity against *Bacillus subtitles* strains in pilot study with inhibition zone diameter of 13mm to 21mm while other pathogens like *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Proteus mirabilis* produce no zone of inhibition

From this study it was observed that the leaves of *Raphanus sativus* possess significant anti microbial activity.[27]

Rama Koyyati et. al., synthesised the Silver nanoparticles from *Raphanus sativus var. Longipinnatus* aqueous leaf extract using silver nitrate solution then this prepared nanoparticles are characterized by using UV-vis spectroscopy, FTIR spectroscopy, SEM and TEM, Energy x- ray diffraction spectra and XRD.

The antibacterial activity of synthesised silver nanoparticles was determined using Disc diffusion method against Gram negative (*Pseudomonas putida, Klebsiella pneumonia*), Gram positive (*Staphylococcus aureus, Bacillus subtilis*) bacteria and zone of inhibition was measured in mm and are as follows *Pseudomonas putida* – 8mm, *klebsiella. Pneumonia* – 9mm, *Staphylococcus aureus* – 10mm, *Bacillus subtilis* – 9mm and these results were compared with Ampicillin and 1mM silver nitrate.

The results indicated that silver nanoparticles synthesised from *Raphanus sativus var. Longipinnatus* leaf extract shows anti bacterial activity against Gram negative and Gram positive bacteria.[28]

Syed sultan Beevi et.al. studied that Acetone and hexane extract of root, stem and leaf extract of *Raphanus sativus* were investigated for their antibacterial activity against food borne and resistant pathogens such as *Bacillus subtitlis, Staphylococcus epidermidis, Enterococcus faecalis, Salmonella typhimurium, Enterobacter aerogenes, Enterobacter cloacae and Escherichia coli.*

Total and individual isothiocyanate components and their relationship with antibacterial activity of *Raphanus sativus* were also evaluated.

Gas Chromatographic analysis revealed the presence of variable amounts of five ITC such as Allyl isothiocyanate (AITC), phenyl isothiocyanate (PITC), benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC) and 4-(Methylthio)-3-butenyl isothiocyanate (MTBITC).

Antimicrobial activity of extracts assessed by agar-well diffusion method.

Minimum inhibitory concentration of hexane and acetone extracts of were determined by tube broth dilution assay. In leaf extract entire range of micro organism were susceptible to acetone extract, whereas hexane extract from leaf showed considerable inhibitory activity only against *Sa. typhimurium* with maximal zone of inhibition of 17.52mm and weak activity against the remaining bacteria.

Acetone extract of leaf showed considerable antibacterial activity against *St. epidermidis* and *St. aureus* with MIC of 0.064 mg/mL, while the MIC was in the range of 0.128-1.02mg/mL for remaining pathogens. Hexane fraction of leaf showed MIC in the range of 0.512-1.02mg/mL for most studied organism.

From the above study, acetone appeared to be ideal solvent for extracting different types of isothiocyanates with appreciable antibacterial activity.

Antibacterial activity of *Raphanus sativus* seemed to be influenced by AITC, PITC and BITC for all organisms expect for *Enterobacter faecalis,* whose inhibitory effect was more related to MTBITC.[29]

3. Antioxidant activity

Kum kum Agarwal, et.al., have found the radical scavenging ability as well as biochemical analysis of different extracts of *Raphanus sativus L.* Was undertaken both the alcohol and water extract of *Raphanus sativus L.* was taken to study the antioxidant activity and biochemical analysis.

The antioxidant activity of the extract was measured by DPPH radical scavenging assay. DPPH is stable nitrogen centered free radical the colour of which changes from violet to yellow upon the reduction by either the process of hydrogen or electron donation. IC50 value of ascorbic acid used as standard reference compound was compared with ethanolic leaf extract, Results revealed that the leaf of *Raphanus sativus L* .has potent antioxidant ability of 78.17% at 200microgram/ml concentration and IC50 Value at 122.33microgram/ml concentration.

From the study it can be observed that ethanolic extract of *Raphanus sativus L*. Leaves has potent antioxidant ability and it can act as radical scavengers for free radicals.[30]

P vanitha Reddy, et.al., studied the antioxidant activity of methanol, ethanol, water extracts of *Raphanus sativus* leaves were investigated or analysed by DPPH radical scavenging assay, reducing power and invitro inhibition of lipid oxidation and these results are compared with standard BHT, alpha tocopherol and ascorbic acid.

The extracts showed a dose dependent scavenging of free radical, DPPH. Ethanolic extract showed higher activity than Methanolic extract and water extract.

Results from this study indicate that, *Raphanus sativus* leaves are rich source of a number of antioxidant compounds like polyphenols, alpha tocopherol, ascorbic acid, beta carotene, glutathione, flavonoids and possess potent antioxidant properties.[31]

Xiaoping Luo et. al., performed activity and investigated that protective effects of radish leaves extract on the oxidative damage in human fetal lung fibroblast (MRC-5) cells. DPPH radical scavenging assay and reducing power assay was performed to evaluate the antioxidant activity and TPC (Total polyphenol content) also measured.

The radish leaves extract shows the best free radical scavenging ability, the highest TPC and the most potent protective effect on hydrogen peroxide induced oxidative damage in MRC-5 cells by decreasing ROS production.[32]

4. Anti hypertensive activity

Da-Hee Chung, et.al., studied the effect of ethyl acetate extracts of radish leaves on hypertension in 11 week old spontaneously hypertensive rats (SHRs) was studied and six wister rats were used as normotensive controls.

The systolic blood pressure (SBP) of the SHRs showed a decreasing trend with the consumption of the radish leaf extract. The SBP of the group fed 90mg extract/kg body weight reduced from 214 mmHg to 166 mmHg and was significantly lower than that of the normotensive and hypertensive controls. The extract increased the concentration of NO in serum and the activities of antioxidant enzymes such as glutathione peroxidase and catalyse in red blood cells.

It was observed that consumption of an ethylacetate extract of radish leaves may have antihypertensive effects in SHRs since it increases the serum concentration of NO and fecal concentration of Na+ and enhance antioxidant activity.[33]

5. Anti inflammatory activity

Hye-jin park et. al., reviewed that *Raphanus sativus L.* Leaves were extracted using an n-hexane, chloroform, ethyl acetate, n-butanol and water soluble fractions. The extract activity on RAW264.7 cells was studied using the CCK- 8 assay.

The Chloroform, ethyl acetate and water *Raphanus sativus L* fractions significantly inhibited the LPS induced NO production in a concentration dependent manner among these fractions chloroform fraction showed the strongest inhibitory effects and hence was used for the further study.

It was found that *Raphanus sativus* leaves extract significantly inhibits COX-2 protein expression in a concentration dependent manner, as well as reduced PGE2 production and Thus mechanism of anti inflammatory effect was by inhibition of PEG 2 production via inhibition of COX2 protein expression. The study found that chloroform fraction suppressed LPS induced production of pro inflammatory mediators in RAW264.7 microphages via modulation of the signal transduction cascades and inhibited the release of several

inflammatory mediators from the macrophages, including iNOS, COX2 and Pro-inflammatory cytokines.[34]

6. Anti ulcer activity

V.C. Devaraj, et.al., have studied that Ethanolic extract (ERS), Petroleum ether fraction (PERS), Chloroform fraction (CRS), Ethyl acetate fraction (EARS) extracts of *Raphanus sativus L*. Leaves and Male albino wistar rats were used for the study.

The study on anti ulcer activity was done by two methods

Acetic acid induced ulcer model - ERS, CRS, EARS & AQRS have offer significant protection against acetic acid induced ulcer. This maybe due to decreased acid secretion, increased mucus secretion or decreased GI motility in case of ERS & AQRS, where as the CRS maybe acting through decreasing the acid gastric motility.

Pylorus ligation induced gastric ulcer method - The ERS, EARS & AQRS reduced the volume of gastric acid secretion, indicating anti secretory effect.

The ERS & AQRS were effective in both models of peptic ulcer disease but CRS was effective only in healing chronic gastric ulcers. Hence the consumption of the leaves of *Raphanus sativus i*n maybe beneficial in peptic ulcer healing.[35]

7. Anti diabetic activity

Mohammed Kawser Het.al., have proved that Anti diabetic activity of *Raphanus sativus* leaf extract in high fat diet and low dose streptozotopin induced diabetes mellitus using female spraque -dawley rats. The hydroalcoholic (70%) extract of radish leaves was prepared by cold maceration method.

The gravimetric analysis for the above extract indicated the presence of flavonoids 5.2%, cardiac glycosides less than 2%, saponins 24.52% and tannins 2.91%. Further HPLC method indicated the presence of rutin 0.12% and myricetin 0.03%.

Rutin and myricetin are reported to possess antidiabetic activity.[36]

Mohammed Kawser H have studied that *Raphanus sativus* leaves possess intestinal alpha-glucosidase inhibitory activity.[37]

Deepti Kaushalkumar J et.al., reported that Antihyperglycemic activity of *Raphanus sativus* extract was found to be as good as the metformin treatment in diabetic rats suggesting that the natural constituents could have act separately or synergistically to induce the hypoglycemic effect.

Thus this study provides the evidence that extract of *Raphanus sativus* ameliorates metabolic abnormalities associated with diabetes and can retard the risk of complications due to chronic hyperglycemia.

The beneficial effect of *Raphanus sativus* extract in diabetes seem to be attributed to the synergistic effect of its bioactive compounds such as flavonoids, glycosides and saponins.[38]

CONCLUSION

The nutrient contents of radish leaves are known to have promising vital phytoconstituents and pharmacological activities.This review can be utilised in the discovery of novel drug molecules with higher efficacy towards drug targeting with lesser side effects.

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