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Phytochemical and Pharmacological Investigations of *Ricinus communis* Linn.

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Abstract: Medicinal plants have always played a vital role for the healthy human life. The family Euphorbiaceous is a family of flowering plants and contains nearly about 300 genera and 7,500 species. Amongst all, the species *Ricinus communis* or castor plant has high traditional and modern medicinal values. The individual parts of the plant like the seed, seed oil, leaves and the roots showed their importance in pharmacology. Traditionally, the plant has been used for the treatment of various diseases in traditional or folk remedies throughout the world. In modern pharmacology, this plant is reported to possess antioxidant, anti-inflammatory, anti-diabetic, central analgesic, antitumor, anti-nociceptive, antiasthmatic activity and other medicinal properties. These activities of the plant are due to the presence of important phytochemical constituents like flavonoids, glycosides, alkaloids, steroids, terpenoids etc. The aim of present article is to explore the chemical constituents, their structures and medicinal importance of *Ricinus communis*.

Keywords: *Ricinus communis;* Euphorbiaceae; Phytochemical Constituents; Pharmacology

I. Introduction

The species *Ricinus communis* Linn belongs to family Euphorbiaceae, monotypic genus, *Ricinus*, and subtribe, Ricininae.[1,2] This plant is popularly known as 'castor plant'. The other common names of this plant in different Indian languages are Jada, Gaba (Oriya); Bherenda (Bengali); Endi, Arand, Erand, Andi, Rend (Hindi); Erand (Marathi); Gandharvahasta, Vatari, Rubu, Urubu, Pancangula, Citra (Sanskrit); Haralu, Oudala, Gida (Kannada); Aran, Banangir (Kashmiri); Erandio, Erando (Guajrati); Arind (Punjabi).[2-4] It is found throughout the country and widely cultivated in the tropics and warm regions.[5,6] This is a fast-growing, perennial shrub or soft wooded small tree up to about 6 meters in height. This plant is cultivated for leaves, flowers or oil production and it grows wild in waste places. In the Indian system of medicine, different part of plants has been used for the treatment of different diseases.[1-7] It is also used as a lubricant, lamp fuel, a component of cosmetics, and in the manufacture of soaps, printer's ink, plastics, fibers, hydraulic fluid, brake fluid, varnishes, paints, embalming fluid, textile dyes, leather finishes, adhesives, waxes, and fungicides.[7]

In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders.[1] The plant has been found to be useful in hepatoprotective[8], antifilarial[9], antioxidant[10], antiasthmatic[11] and antimicrobial[12] activities. The root of this plant is also useful as an ingredient of various prescriptions for nervous diseases and rheumatic affections such as lumbago, pleurodynia and sciatica.[13] Roots of this plant showed anti-inflammatory and free radical scavenging[14], anti-fertility[15], anti-diabetic[16], and antimicrobial[17] properties.

In the following section, a comprehensive coverage of the literature covering the developments in the field of isolation of phytochemicals, the traditional and modern pharmacological applications and toxicological studies of ricin is presented.

II. Phytochemical Investigation

Phytochemicals are the compounds that are isolated from plant kingdom. The composition of these chemicals is dependent on their geographical locations and harvesting conditions. Hence, their quantitative and qualitative investigations are necessary to understand their applications. *R. communis* has wide range of applications. The phytochemists have worked tirelessly to find chemical constituents present in different parts of the plant. A homologous long chain 1,3-alkanediols and 3-hydroxyaldehydes have been obtained from the leaf cuticular waxes of this plant by Vermeer et al.[18] The cuticular waxes also comprised of alkanes, primary alcohols, aldehydes, fatty acids and triterpenoids.[18] Kang *et al* identified various alkaloids and flavonoids in the leaves of this plant.[19] The structures of these compounds were determined through spectroscopic analysis, chemical correlation and chemical degradation studies. A list of phytochemicals obtained from different parts is given below.

II.1 Leaves: Aldehydes (C_{26} and C_{28})[18], Alkanes (C_{26}-C_{29})[18], α-Amyrin (**1**)[18], β-Amyrin (**2**)[18], *N*-Butylmorpholine (**3**)[20], Chlorogenic acid (**4**)[21], Camphor (**5**)[22], 1,8-Cineole (**6**)[22], Citric acid[23], β-Caryophyllene (**7**)[22], Decanamine[20], *N*-Demethylricinine (**8**)[19,24], Di-butylphthalate[20], 2,5-Dihydroxybenzoic acid (Gentisic acid)[21], β-Eleosteric acid (**9**)[25], Ellagic acid (**10**)[10], (-)-Epicathechin (**11**)[21], Fumaric acid[23], Gallic acid (**12**)[10,21], Hexacosane-1,3-diol[18], 3-Hexen-1-ylacetate[20], Kaempferol (**13**)[19], Kaempferol 3-*O*-β-D-glucopyranoside (Astragalin, **14**)[19], Kaempferol 3-*O*-β-D-xylopyranoside (**15**)[19], Kaempferol 3-*O*-β-rutinoside (nicotoflorin, **16**)[19], Linoleic acid[25], Linolenic acid[25], Lupeol (**17**)[18], Myristic acid (**18**)[26], Malic acid[23], Methyl gallate[27], Neochlorogenic acid (an isomer of **4**)[21], 4-Octadecylmorpholine (**19**)[20], Oleic acid[26], Palmitic acid (**20**)[26], Palmitoleic acid[26], α-Pinene (**21**)[22], Primary alcohols (C_{22}-C_{38})[18], Quercetin (**22**)[19], Hyperoside (**23**)[21], Quercetin 3-*O*-β-Rutinoside (Rutin, **24**)[19], Quercetin-3-*O*-β-D-glucopyranoside (isoquercetin, **25**)[19,27,28], Quercetin 3-*O*-β-D-xylopyranoside (Reynoutrin, **26**)[19,27], Ricinine (**27**)[29-31], β-Sitosterol (**28**)[31], Stigmasterol (**29**)[31], Stearic acid (**30**)[26], Tartaric acid[23], Tannins[21].

II.2 Essential oils from leaves: Camphor (**5**)[32], Camphene (**31**)[32], 1,8-Cineole (**6**)[32], α-Pinene (**21**)[32], α-Thujone (**32**)[32].

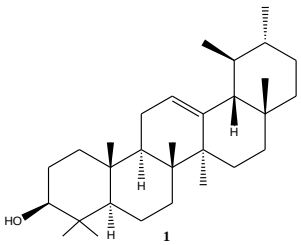
II.3 Roots: 3-*O*-Benzyol-stigmasta-5,22-dien-3β-21-diol (Ricinusterryl benzoate)[33], Dipiperenoyl methyl ester methylene (Ricipiperanyl ester)[33], Erandone (**33**)[34], 3-α-Hydroxypentatriacont-14-en-26-one (Ricipentatriacontanol)[33], Indole-3-acetic acid[35], Lupeol (**17**)[34], 1-Oleio-2-palmitoglyceryl phosphate[33], Quercetin-3-*O*-β-D-glucopyranoside (isoquercetin, **25**)[36], Quercetin 3-*O*-β-Rutinoside (Rutin, **24**)[36], Kaempferol 3-*O*-β-D-[6-O-acetylglucopyranosyl(1$\rightarrow$3)-β-D-galactopyranoside] (Ricinitin)[36].

II.4 Seed: Arachidic acid (**34**)[37], Eicosenoic acid[37], Ergost-5-en-3-ol (**35**)[38], Fucosterol[38], Glycine[39], Hydroproline[39], Leucine[39], Linoleic acid[37], Maltose[39], Oleic acid[37], Palmitinic acid[37], Phenylalanine[39], Probucol[38], Proline[39], Ricinoleic acid (9*Z*,12R)-12-Hydroxyoctadec-9-enoic acid, **38**)[27,40,41], Stearic acid (**30**)[37], Ricin[42], Ricinine (**27**)[39], γ-Sitosterol[38], Stigmasterol (**29**)[38], Tryptophan[39], Valine[39].

III. Pharmacological Applications

III.1 ***Traditional Pharmacological Uses:*** *R. communis* has very high traditional medicinal values. The oil extracted from the seeds of this plant has been used by local people since about 2000 BC.[2,4,43-45] The use of different parts of this plant for the treatment of various diseases in traditional or folk remedies throughout the world has been reviewed.[6] The oil is extracted after removing the hard protective cover of seeds. This oil is also known by other names like ricinus oil or castor oil.[46] This oil has been used in local medicines as a laxative, arthritic diseases and cathartic in Unani, Ayurvedic and other ethno medical systems.[43] The oil acts as an osmotic laxative in mild to moderate constipation. This holds water in the intestines and performs total cleansing of the large intestine.[44] This is one of the safest and most reliable purgatives we possess for the relief of obstinate constipation.[45] In addition, it is a traditional folk medicine used in the treatment of warts, cold tumors, and indurations of mammary glands, corns, and moles.[47-49] The oil is also externally applied and internally taken for sciatica; arthritis; gout and paralysis. The use of hot fomentation is beneficial for any cyst, inflammation, tumour or lump. The oil is beneficial in the treatment of styes, conjunctivitis

and foreign objects in the eyes. The oil is also useful to treat dry skin conditions such as psoriasis and eczema.[44] This is often given orally, alone or with quinine sulphate to induce labour in pregnancy. It is also included in the treatment of piles.[44] The decoction of leaves are heated and applied to a woman's breasts to improve secretion of milk. Decoction is a practice of obtaining dissolved chemicals from herbal or plant materials by first mashing and then boiling in water. The decoction has also been reputed to increase the secretion of milk when administered internally.[50] An infusion of leaves is used for stomache-ache, and as a lotion for the eye. Roots are administered in the form of a decoction for lumbago and allied complaints, in the form of a paste for toothache. In the decoction process, a herbal or plant material is mashed and then boiled in water to extract active compounds. Root bark is reported to be a powerful purgative.[50]



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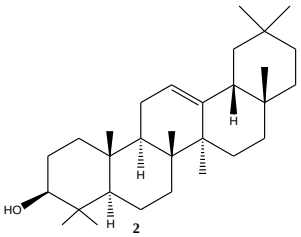

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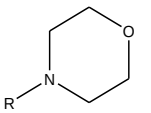
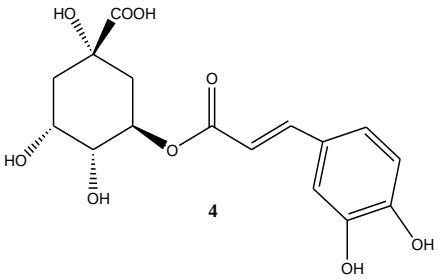


3 R = $(CH_2)_3CH_3$
19 R = $(CH_2)_{17}CH_3$



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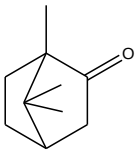

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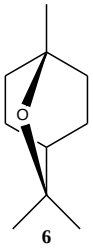

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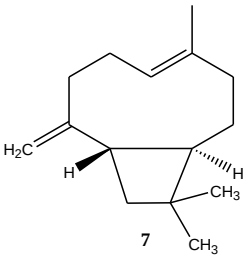

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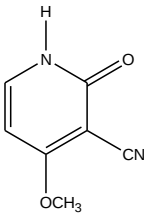
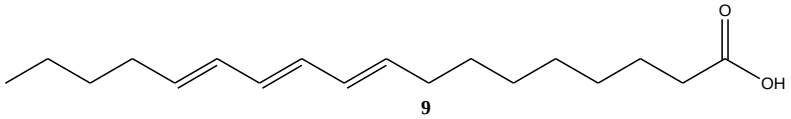


8 R = H
27 R = CH_3



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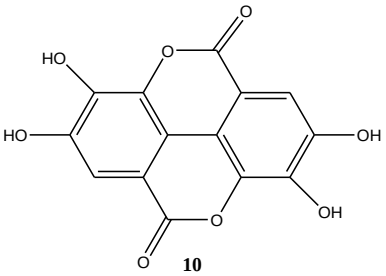

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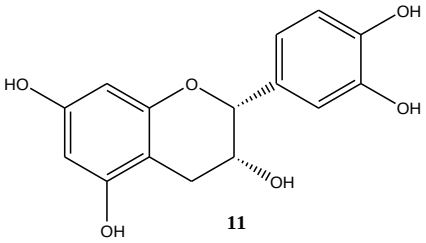

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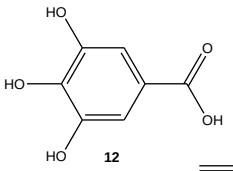

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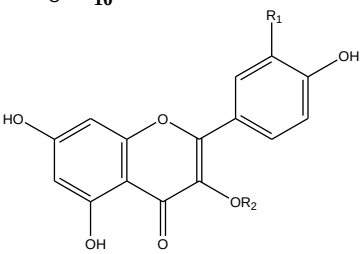
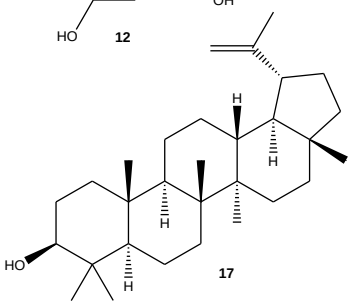


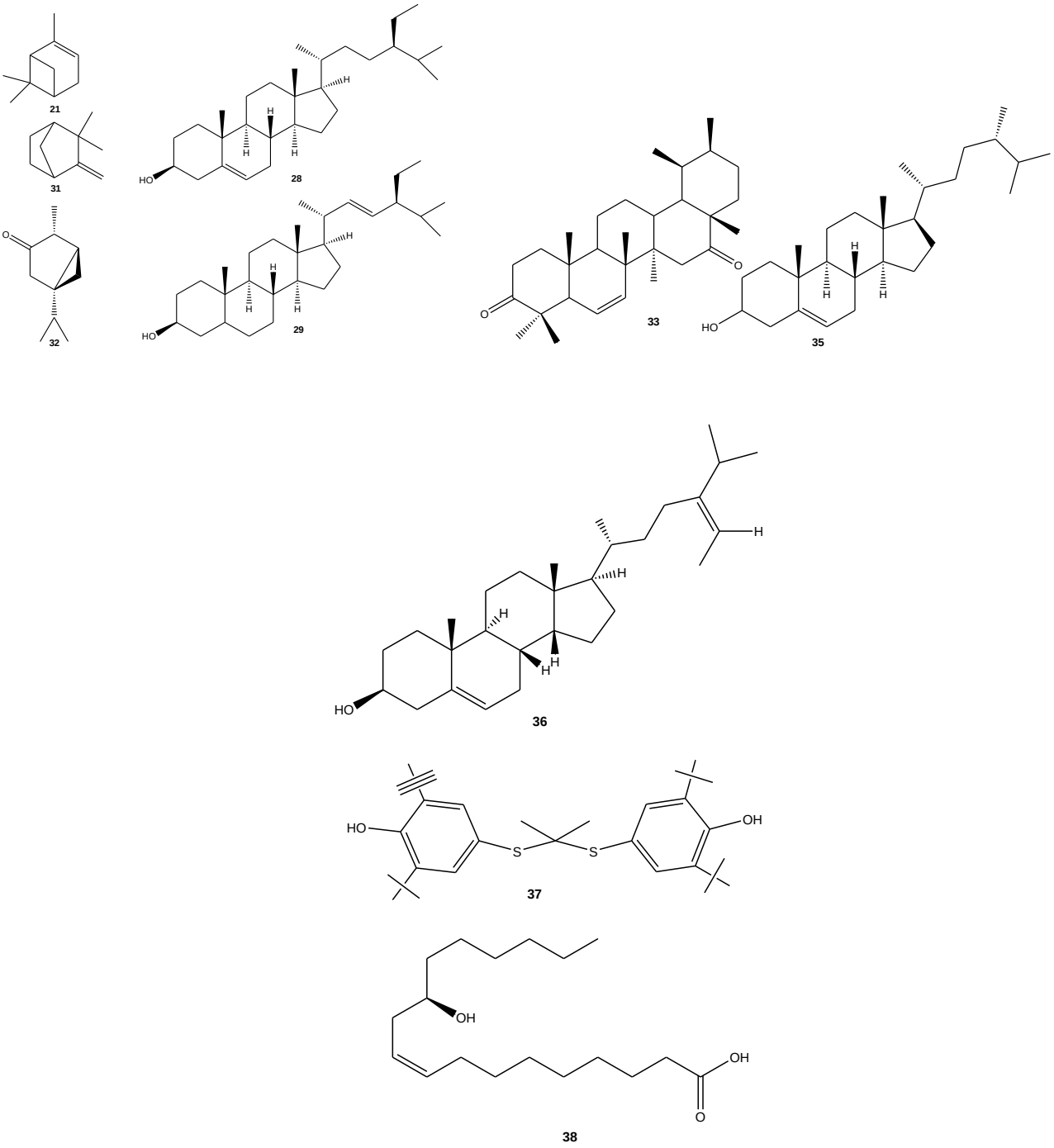
13 R_1 = H; R_2 = H
14 R_1 = H; R_2 = β-D-glucopyranosyl
15 R_1 = H; R_2 = β-D-xylopyranosyl
16 R_1 = H; R_2 = β-rutinosyl
22 R_1 = OH; R_2 = OH
23 R_1 = OH; R_2 = β-D-galactopyranosyl
24 R_1 = OH; R_2 = β-rutinosyl
25 R_1 = OH; R_2 = β-D-glucopyranosyl
26 R_1 = OH; R_2 = β-D-xylopyranosyl



17

$CH_3(CH_2)_nCOOH$

18 Myristic acid	n = 12
20 Palmitic acid	n = 14
30 Stearic acid	n = 16
34 Arachidic acid	n = 18



III.2 *Modern Pharmacological Uses*

III.2.1 Antioxidant activity: Antioxidants are compounds that prevent or delay the oxidation of oxidizable materials by scavenging free radicals. Free radicals are responsible for oxidative stress which promotes the development of chronic degenerative diseases including coronary heart disease, cancer and aging.[51,52] The plant *R. communis* has significant radicals scavenging abilities on 2,2-dipehyl-1-picrylhydrazyl (DPPH)[53], nitric oxide (NO)[54], and superoxide radicals[54]. The CH_3OH:H_2O (8:2) extract of leaves showed strong DPPH radical-scavenging activity.[10] The stem and leave extracts also produce antioxidant activity due to the presence of flavonoids in their extracts.[10]

III.2.2 Antidiabetic activity: Diabetes or diabetes mellitus is a group of metabolic diseases in which a person has high blood glucose (blood sugar). This may be due to inadequate insulin production, or because the body's cells do not respond properly to insulin, or both. An antidiabetic agent controls diabetes. World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects.[55] The

administration for 20 days of 50% ethanolic extract of roots has been found to be effective to the diabetic rats on fasting blood glucose, and also on total lipid profile and liver and kidney functions on 10^{th} and 20^{th} day.[16] The effective dose of 500 mg/kg body weight not only significantly lowered the fasting blood glucose (FBG) of the diabetic animals to almost normal level, but also increased the insulin levels and caused improvement in lipid profile and body weight of the diabetic animals. The extract also caused reversal of the damage of liver and kidneys seen in diabetic animals.[16]

III.2.3 Antimicrobial activity: An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial substances are grouped according to the microorganisms against which they act. The antimicrobial activity of the oil isolated from leaves was investigated in order to evaluate its efficacy against twelve bacteria and four fungi species, using disc diffusion and minimum inhibitory concentration methods.[32,56] The results are comparable to the antibiotic ampicillin, used as a positive control. The isolated leaf oil showed strong antimicrobial activity against all microorganisms tested with higher sensitivity for *Bacillus subtilis*, *Staphylococcus aureus* and *Enterobacter cloacae*.[32] These findings showed that the variation in quantities of the main components such as camphor (**5**) and 1,8-cineole (**6**), might be responsible for the different antimicrobial activity.[32] The MIC and IC_{50} values of essential oil on bacteria ranged from 120 µg/ml to 300 µg/ml, and from 210 µg/ml to 870 µg/ml, respectively, whereas on fungi the value ranged from 140 µg/ml to 250 µg/ml and from 350 µg/ml to 590 µg/ml.[32] Naz et. al. studied the *in vitro* antimicrobial properties of methanol (CH_3OH), ethanol (C_2H_5OH) and water (H_2O) extract of leaves against gram positive and gram negative bacterial strains and *Aspergillus flavus* and *A. fumigatus* fungal strains.[57] Methanol extract was found to be more efficient than the other two extracts.[57] The methanol and ethyl acetate ($CH_3COOC_2H_5$) extracts of leaves showed good activity against *P. aeruginosa*, *S. aureus*, *K. Pneumonia* and *Proteus vulgaris.* The antimicrobial assay revealed that these extracts possess good zone of inhibition.[58] The secondary infections in the immune compromised oral cancer cases were due to bacterial and fungal species. The co-administration of *R. communis* with the immune suppressant drugs for the prevention of infection against oral cancer treatment patient showed significant result.[59]

III.2.4 Anti-Inflammatory activity: Inflammation is a localized response that produces redness, warmth, swelling and pain due to infection or injury. In the Indian system of medicine, the leaves, roots and seeds have been used for the treatment of inflammation.[14,60,61] Methanolic extract of the root was studied for anti-inflammatory activity in carrageenan induced hind paw edema model in Wistar albino rats.[62] Ricinine (**27**), Quercetin (**22**) and *n*-butanol soluble fraction of methanol extract gave promising result for anti-inflammatory activity.[62] Root crude methanolic, enriched *n*-hexane fraction isolates at doses 100 mg/kg p.o. exhibited significant ($P < 0.001$) anti-inflammatory activity in carrageenan-induced hind paw oedema model.[34] The compound ricinoleic acid (**38**), the main component of castor oil also showed remarkable analgesic and anti-inflammatory effects.[41] The results showed that **38** may be seen as a new capsaicin-like, non-pungent anti-inflammatory agent suitable for peripheral application.[41]

III.2.5 Antinociceptive activity: Nociception is a physiological term to describe neural process of encoding and processing noxious stimuli.[63] The methanol extract of the leaves possesses antinociceptive activity in acetic acid induced writhing test, formalin induced paw licking and tail immersion test in mice.[64] The extract showed analgesic effect at a dose of 150 mg/kg on formaline induced pain corresponding to neurogenic and inflammatory pains.[64] The extract also increases latent time after 90 minutes of drug treatment in tail immersion model.[64]

III.2.6 Anti-fertility activity: The seed extract have been found to possess anti-fertility activity. The ether soluble portion of the methanol extract of seeds when administered subcutaneously to adult female rats and rabbits showed anti-implantation and anti-conceptive activity.[65] The extract protected the animals from getting pregnant for over three gestation periods.[65] Further, the extract did not show any long term effect on the pups that were born after the extract effect.[65] The seed extract was found to possess anti-implantation and abortificient effects. It was also observed that the seed extract prolonged the oestrus cycle of guinea pigs. The dioestrus phase was significantly prolonged as well. After stopping the administration of the extract, the normal dioestrus phase and oestrus cycle started to resume. The seed extract also reduced the weight of the uterus without affecting that of the ovaries significantly. The anti-fertility effect of *R. communis* in female guinea pigs might be extrapolated to human beings.[66] The 50% alcohol extract of the roots possess significant reversible anti-fertility effect.[67] There was a drastic reduction in the epididymal sperm counts in male rats. The extract also

caused changes in the motility, mode of movement and morphology of the sperms. The reductions in the fructose and testosterone levels further suggested the reduced reproductive performance.[67]

III.2.7 Anti-hepatotoxicity: The hepatoprotective activity is the ability to protect liver damage.[68] Liver cirrhosis and drug induced liver injury are major health problem in western and developing countries.[69] Herbal and Plant products are popular and potential hepatoprotective agents.[70,71] An ethanol extract of the leaves showed significant protection against galactosamine-induced hepatic damage.[72] The maximum activity was obtained in the butanol fraction of the ethanol extract. Further purification led to the isolation of two active compounds ricinine (**27**) and N-demethylricinine (**8**). The compound **8** was found to be more active than **27**.[72] In another study by Padmapriya et al [8], ethanolic leaf extract was evaluated at a dose of 100 mg/kg body weight against Ketoconazole (Phytoral) induced liver damage in mice. The result showed relative significant reduction in hepatic enzymes of treated mice and confirmed the traditional uses of this plant as a potential hepatoprotective agent.[8] Pingale *et al* used the powder of leaves against hepatosuppression induced by carbon tetrachloride (CCl_4). It was found that the powder had high potential in healing liver parenchyma and regeneration of liver cells. It showed best ability to protect liver and may act in humans even as potent liver tonic due to the presence of large number of antioxidants.[73] Natu *et al* also studied the protective effect of leaves in experimental liver injury caused by carbon tetrachloride in albino rats.[74] They reported the pharmacological effects of the whole leaves, cold aqueous extract and a glycoside extracted from the leaves. The whole leaves provided protection against liver necrosis while the cold aqueous extract provided protection only against fatty changes.[74] The glycoside protected the liver from cell necrosis. The leaves had significant parasympathetic activity and parasympathetic predominance can be expected to cause an increase in blood supply to the liver and protection against hepatotoxic agents.[74]

III.2.8 Cytotoxic Activity: Cytotoxicity is the ability of any substances to be toxic to cells. Ricin is a heterodimeric protein isolated from the seeds. It possesses cytotoxic activity by virtue of its ability to fatally disrupt protein synthesis.[75] Therapeutically, it can be used to specifically target and destroy cancer cells.[75] The leaves on the other hand, have another range of cytotoxic phytochemicals which induces apoptosis *via* translocation of phosphatidyl serine to the external surface of cell membrane and loss of mitochondrial potential. These compounds included three monoterpenoids: camphor (**5**), 1,8-cineole (**6**) and α-pinene (**21**) and a sesquiterpenoid: β-caryophyllene (**7**).[76] The *R. communis* agglutinin I (RCA I), was found to preferentially binds to and is internalized by tumour endothelial cells leading to VEGFR-2 down-regulation, endothelial cells apoptosis and tumour vessel regression. It has no effect on normal blood vessels.[77] A volatile extract from the leaves was cytotoxic to several human tumor cell lines in a dose-dependent fashion.[76] Apoptosis was induced in SK-MEL-28 human melanoma cells at a concentration of 20 μg mL^{-1}. Translocation of phosphatidyl serine to the cell membrane's external surface and loss of mitochondrial membrane potential have also been detected with this extract.[76] The effect of different concentrations of essential oil from leaves on HeLa cell survival was also studied. The cytotoxicity of the oil was quite strong with IC_{50} values less than 2.63 mg/mL for both cell lines.[32] Ricin A[78], a lectin isolated from *R. communis* possess antitumor activity, it was more toxic to tumor cells than to non-transformed cells, judged from the ED_{50} of the lectin towards tumor cells and non-transformed cells.[79]

IV. Toxicological Analysis

The seed from *R. communis* contains two toxins that are poisonous to humans, animals and insects.[80-84] One of the main toxic proteins is ricin which is a potent cytotoxin but a weak hemagglutinin, whereas the other one RCA (*Ricinus communis* agglutinin) is a weak cytotoxin and a powerful hemagglutinin.[86] Ricin works by inactivating the ribosomes present in cells. Due to this the ribosomes do not produce proteins. Cells need these proteins to survive and reproduce, so when ribosomes are inactivated, cells die. The seed poisoning by ingestion is due to ricin, not RCA, because RCA does not penetrate the intestinal wall. The RCA does not affect red blood cells (RBCs) unless given intravenously. If RCA is injected into the blood, it will cause the red blood cells to agglutinate and burst by hemolysis.[83] The effect of ingestion of castor bean in a puppy was studied by Mouser et al.[80] Ingestion of masticated seeds resulted in high morbidity, with vomiting and watery to hemorrhagic diarrhea. The prognosis varied with the number of seeds ingested, the degree of mastication, individual susceptibility and the delay in treatment. Despite supportive therapy, the puppy

died several hours after presentation for acute vomiting, diarrhea and lethargy. Histopathologic findings included superficial necrotizing enteritis of jejunum and occasional, random foci of coagulative necrosis in the liver. Ricin toxicological effect was confirmed by liquid chromatography/mass spectrometry using Ricinine as a marker.[80] Coopman *et al* studied the suicidal death of a 49 year-old man who committed suicide by intravenous and subcutaneous injection of a castor bean extract.[81] The patient developed nausea, vomiting, diarrhea, dyspnoea, vertigo and muscular pain after taking injection. Despite symptomatic and supportive intensive care, he died due to multiorgan failure.[81] It is the first time that ricin had been identified in a case of castor bean poisoning. Based on the clinical symptoms and the results of the toxicological analysis, it was concluded that the death was caused by intoxication with plant toxins originated from the plant.[81]

V. Summary and Future Prospects

Traditional medicines are always at the centre of attention to cure various ailments. *R. communis* is native plant of India and all the parts of this plant have been medicated. Various crude fractions and purified components have shown potential medicinal and pharmacological activities. The antioxidant and free radical scavenging activities of phyto-components isolated from this plant give us an impression that the plant might be the future drug for diversified panel of tumors and cancers. The plant is also reported to possess anti-diabetic, antimicrobial, anti-inflammatory, anti-nociceptive, anti-fertility, anti-hepatotoxicity and other medicinal properties. These activities of the plant are due to the presence of important phytochemical constituents like flavonoids, glycosides, alkaloids, steroids, terpenoids etc.

A systematic scientific approach from phytochemicals either in pure or crude form to modern drug development can provide valuable drugs from traditional medicinal plants. Development of such medicines with international safety and efficacy can give better and satisfactory treatment of various diseases. To ensure ample production of phyto-constituents with in limited space and time, new approaches must be adopted. This is because the prospecting of bioresources for economic development is emerging as a new economic venture.

VI. References

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