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Phytochemical Screening and Mass Spectral Analysis of *Azadirachta indica. Linn.* Gum

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ABSTRACT

Azadirachta indica is a member of the Meliaceae family, is well-known for its therapeutic properties. Neem gum is a commercially available product that is used in a variety of industries. The crude Neem gum is collected in this study, with water as the purifying solvent and ethyl alcohol as the precipitating agent. Secondary metabolites have been screened in neem gum. FTIR spectroscopy is used to identify the functional biomolecules in the gum. According to mass spectral analysis, fatty acids make up 50% of neem gum. The antibacterial activity of gum against several bacterial pathogens was tested using the disc diffusion method. The gum is more active and reveals that it can be utilised to treat antibiotic-resistant illnesses as an alternative to antibiotics. These findings reveal that the gum in question may be used to create a novel medicinal medication.

Keywords: Neem gum, Gas chromatography, Bioactive compounds.

INTRODUCTION

Due to its use in the treatment of many ailments[1], Neem (*Azadirachta indica*), a Meliaceae plant found in India, Pakistan, Bangladesh, and Nepal, has therapeutic implications in illness cure and formulation[2]. Although many chemicals are extracted from all parts of the Azadirachta plant, some chemicals extracted are tested for their pharmaceutical activities[2]. Quercetin and ß-sitosterol, two polyphenolic flavonoids extracted from fresh neem leaves, have antifungal and antibacterial properties[3]. The oil from Neem seed kernels contains a bitter crude component called Nimbidin have many biological actions[4]. Tetranortriterpenes such as Nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid are all these components obtained from this crude principle. Nimbidin and sodium nimbidate both have strong anti-inflammatory action against carrageen-induced acute paw oedema and formalin-induced arthritis in rats[5,6]. As evidenced by its ability to stop Tinea rubrum from growing, Nimbidin also has antipyretic and antifungal properties. Nimbidin has been shown to totally inhibit the

growth of Mycobacterium TB[7,8]. Since 1959, nimbidin and nimbin have been shown to have spermicidal action in rats and humans[9]. Nimbolide inhibits the growth of Plasmodium falciparum, indicating antimalarial activity[10]. Nimbolide acts as an antimicrobial agent and shows high activity against *Staphylococcus aureus* and *Staphylococcus coagulase*[11]. *Azadirachta* seed oil contains a compound named Gedunin that has good antimicrobial and antimalarial properties[12]. A highly oxygenated compound *Azadirachtin*, isolated from the seed of neem tree shown to have a substantial antifeedant effect, has also been shown to have antimalarial activity. It prevents the development of malarial parasites[13]. Mahmoodin, a deoxygedunin derived from seed oil, was found to have moderate antibacterial activity against various human pathogenic bacteria strains[14]. Gallic corrosive, (+) gallocatechin, (-) epicatechin, (+) catechin, and epigallocatechin are found in consolidated tannins from the bark, and gallic corrosive, (-) epicatechin, (+) catechin, and epigallocatechin are fundamentally answerable for restraining the age of chemiluminescence by enacted human polymorphonuclear neutrophils[16], inferring that these mixtures repress PMN oxidative burst during irritation. For most bug orders, azadirachtin is a taking care of inhibitor and development disruptor. The seeds of the neem tree*, Azadirachta indica*, contain it[17,18]. *Azadirachta indica* (Neem) has been utilized to treat a wide scope of sicknesses since antiquated times. Its parts (green leaves, ready organic products, and their jackets, Neem seed, root, bark, uncrushed twigs, stem bark, root bark, gum, new entire organic product, and dry leaves) have been utilized to treat a wide scope of illnesses since antiquated times.

The gum exudate from the stem of the *Azadirachta indica* tree (Neem tree) is a boring, dissolvable regular exudate that reaches in shading from dazzling yellow to golden[19]. Proteins and polysugars are combined as one. The proteins are firmly bound to the polysaccharides, which are the primary constituents. Because of this intricacy[20], the underlying clarification of proteins and polysaccharides from neem gum is assorted. D-glucoronic corrosive, L-arabinose, L-fucose, mannose, xylose, rhamnose, D-glucosamine, aldobiuronic corrosive, serine, threonine, and aspartic corrosive have all been found in neem gum[21]. Moreover, it was found to contain natural unsaturated fats[22]. *Azadirachta indica* gum is utilized in an assortment of areas for business purposes. It's used in beauty care products (facial covers, moisturizers, and face powder)[23], paper (cement and paper fortifying)[24], drugs (sterile creams, tablet folio, and coater)[25], materials (texture coloring and printing)[24], and food (balancing out specialist, gels, and thickening specialists)[26]. A review of the writing uncovers the presence of various synthetic substances; however, no endeavors have been made to disconnect them. Thus, the current review plans to look at the phytochemical constituents with GC-MS examination zeroing in exclusively on the detachment and likely exercises of neem gum on pathogenic microbes, to contrast them with past investigations and decide their appropriateness for use in food and drugs businesses as normal important items.

MATERIAL AND METHODS

Collection and Purification of Gum

The neighbourhood was scoured for unrefined Neem gum (NG). E. Merck provided the ethyl liquor (India). Unrefined gum was broken down in sufficient cleaned water and warmed to a temperature of 40°C. To eliminate the undissolved part of the gum arrangement, it was sifted through a twofold overlap of muslin texture following two hours. Ethyl liquor was utilized to encourage the gum, which was then dried in a 40°C stove. The purified neem gum were then kept up within a desiccator for five days.

Phytochemical analysis

The phytochemical component of neem gum was identified using a conventional procedure.

FTIR Spectroscopy Analysis

The IR spectra of the material were acquired using the KBr disc technique on a Schimadzu FTIR–470 infrared spectrophotometer in the range of 4500 cm^{-1}–400cm^{-1}.

GC-MS analysis

The accompanying settings were utilized for GC-MS investigation on a Perkin Elmer GC Clarus 500 framework with an AOC-20i autosampler and gas chromatograph interacted

to a mass spectrometer (GC-MS): At the center of the Elite-1 intertwined silica hairlike section (30 x 0.25 mm ID x 1M df, 100 percent Dimethyl poly diloxane), working in electron sway mode at 70 eV, helium (99.999 percent) was used as a transporter gas at a persistent progression of 1 mL/min and an infusion volume of 0.5 L was utilized (split proportion of 10:1). The temperature of the injector is 250°C, and the temperature of the particle source is 280°C.

Antibacterial movement

The gum's antibacterial movement was considered in contrast to *Klebsiella pneumoniae* (MTCC 432), *Pseudomonas aeruginosa* (MTCC 420) (*Gram-negative* microbes), *Staphylococcus aureus* (MTCC 497), *Bacillus cereus* (MTCC 430), and *Enterococcus faecalis* (MTCC 439) (*Gram-positive* microorganisms). The Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, gave these strains. The stock culture was kept at 37 degrees Celsius on Muller Hinton agar medium (Himedia synthetics).

The circle dissemination technique was utilized to test the antibacterial movement of gum. Independently, the tried bacterial strains were put onto agar plates. The sterile circle holding the gum arrangement was then positioned over the cultivated agar plates so that the zone of hindrance didn't cover. A typical anti-infection, amikacin 5g/plate, was utilized as a bacterium standard. The plates were kept up at room temperature for two hours to permit the gum to diffuse into the agar, then, at that point, hatched at 37 degrees Celsius for 24 h for the bacterial strain. After the brooding time frame, the plates were inspected for the presence of a zone of restraint (ZI), which was evaluated in millimeters (mm). The Activity Index was created utilizing the accompanying equation in view of the outcomes.

$$Activity\ Index\ (AI) = \frac{Inhibition\ Zone\ of\ the\ sample}{Inhibition\ Zone\ of\ the\ standard}$$

RESULT AND DISCUSSION

Phytochemical analysis

Table 1 indicates secondary metabolites such as tannins, alkaloids, phenols, phytosterols, xanthoproteins, carboxylic acids, saponins, and carbohydrates as well as the phytochemical analysis of the gum in water.

Table 1: AIG's phytochemical analysis (G2)

Sl. No	Tests	AIG
1	Carbohydrates	+
2	Proteins	_
3	Alkaloids	+
4	Phenols	+
5	Flavonoids	_
6	Phytosterols	+
7	Quinones	-
8	Xanthoproteins	+
9	Coumarins	_
10	Carboxylic acids	+
11	Saponins	+
12	Tannins	+

FTIR Spectroscopy Analysis

The practical gathering found in the gum was approved utilizing Fourier change infrared spectroscopy (FTIR). Fig. 1 shows the FTIR Spectra of Neem Gum (G2), which uncovers a huge and strong top at 3417 cm^{-1} because of hydrogen-fortified OH extending of alcohols and phenols. The C-H extending, which is credited to the presence of alkenes, is demonstrated by the top at 2924 cm^{-1}. The N-H twisting and C=O extending of amide linkages are addressed by a serious and thin top at 1627 cm^{-1} and 1728 cm^{-1}, separately; this is relied upon to be because of the proteinaceous substance of neem gum, i.e., amino acids present in gum like alanine, asparagine, glycine, and so forth, and sugars present like fructose, galactose, and so on. The C-O holding of liquor is portrayed by a little top at 1041 cm^{-1}.

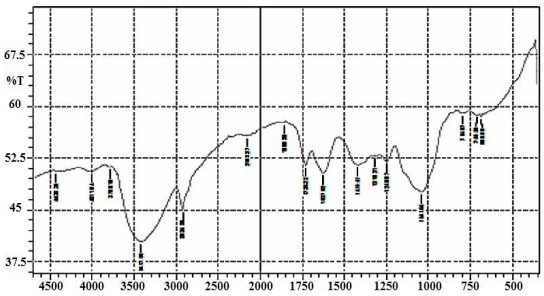

Fig. 1. AIG's FTIR Spectrum (G2)

GC-MS analysis

The presence of ten fixings might be found in the GC-MS spectra of neem gum. Table 2 records the elements of neem gum, and Fig. 2 portrays the total mass range of neem gum. 4-Butyl-1-thia-cyclohexane, Z, Z-3,13-Octadecedien-1-old, Estra-1,3,5(10)-trien-17-one, 3-methoxy-17-methoxime, Adipic corrosive, cis-non-3-enyl ethyl ester, and 2,6-Lutidine, 3,5-dichloro-4-dodecylthiol are the significant constituents present in neem gum.

Table 2: AIG's Constituents (G2)

S.No	Retention Time	Compound Name	Mol. Formula	Mol. Wt.	Area %
1	38.59	4-Butyl-1-thia-cyclohexane	$C_9H_{18}S$	158	22.85
2	38.60	6-Bromohexanoic acid, isopropyl ester	$C_9H_{17}BrO_2$	236	22.53
3	38.747	Adipic acid, cis-non-3-enyl ethyl ester	$C_{17}H_{30}O_4$	298	22.23
4	38.765	Bicyclo[4.1.0]heptan-2-OL	$C_{10}H_{18}O$	154	19.73
5	38.855	Z,Z-3,13-Octadecedien-1-ol	$C_{18}H_{34}O$	266	16.41
6	38.860	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282	18.62
7	38.985	2,6-Lutidine 3,5-dichloro-4-dodecylthiol	$C_{19}H_{31}Cl_2NS$	375	23.05
8	38.995	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	$C_{12}H_{17}NO_2$	207	20.05
9	39.015	Estra-1,3,5(10)-trien-17-one, 3-methoxy-,17-methoxime	$C_{20}H_{27}NO_2$	313	15.46
10	39.025	3-Methoxy-1,3,5(10)-estratriene-17-methoxime	$C_{20}H_{27}NO_2$	313	19.43

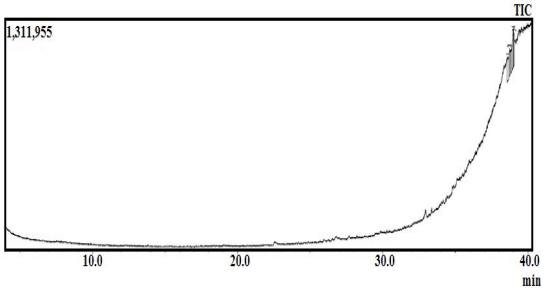

Fig. 2. AIG's GC-MS total Chromatograph (G2)

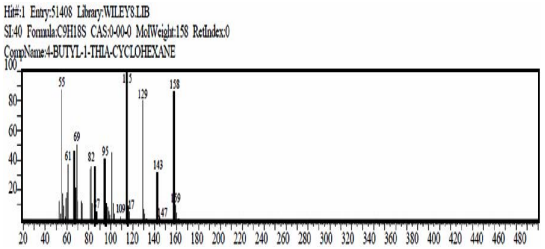

Fig. 3. 4-Butyl-1-thia-cyclohexane EI mass spectrum

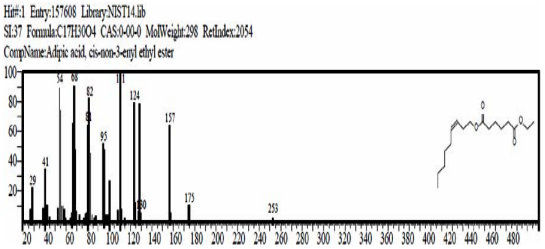

Fig. 4. Adipic acid, cis-non-3-enyl ethyl ester EI mass spectrum

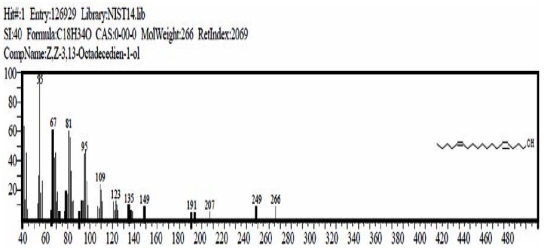

Fig. 5. Z,Z-3,13-Octadecedien-1-ol EI mass spectrum

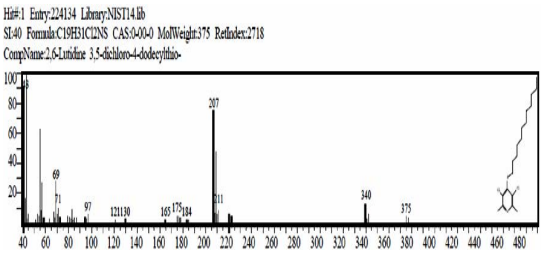

Fig. 6. 2,6-Lutidine 3,5-dichloro-4-dodecylthiol EI mass spectrum

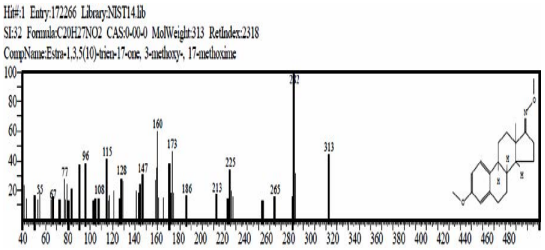

Fig. 7. Estra-1,3,5(10)-trien-17-one, 3-methoxy-, 17-methoxime EI mass spectrum

Figure 3 shows the EI mass range of 4-Butyl-1-thia-cyclohexane. $C_9H_{18}S$ is addressed by the top at m/z 137, which arose at R.T. 38.59 in the all-out mass chromatogram. Fig. 4 shows the EI mass range of Adipic corrosive, cis-non-3-enyl ethyl ester. $C_{17}H_{30}O_4$ is liable for the top at m/z128 with R.T. 38.745. The EI mass range of Z, Z-3,13-Octadecedien-1-old is displayed in Fig. 5. The spectra uncovered a top at m/z 126 with a R.T. of 38.855. It's equivalent to $C_{18}H_{34}O$. The EI mass range of 2,6-Lutidine, 3,5-dichloro-4-dodecylthiol is displayed in Fig. 6. $C_{19}H_{31}Cl_2NS$ is ascribed to the top at m/z 149 with a R. T. of 38.99. Fig. 7 shows the EI mass range of Estra-1,3,5(10)-trien-17-one, 3-methoxy-17-methoxime. $C_{20}H_{27}NO_2$ is addressed by the top at m/z 130, which arose at R.T. 39.02 in the all out mass chromatogram.

Antibacterial activity

The distance across the hindered

development around the wells was utilized to compute the zone of restraint for both *Gram-negative* and *Gram-positive* microorganisms. Fig. 8 portrays the antibacterial movement of Neem Gum against the pathogenic strains researched. Table 3 shows the upsides of the zone of restraint acquired from the measure. It's quite important that every one of the bacterial strains inspected in this study was impervious to Neem Gum. When contrasted with the plant removal, *Gram-positive* microbes showed great awareness. The adequacy of plant-based items as a wellspring of antibacterial synthetic compounds has been illustrated.

Table 3: AIG's Antibacterial activity (G_2)

Name of the Organisms	Zone of the Inhibition (mm)G_2		Control (Nystatin) mm
	ZI (mm)	AI (mm)	
Klebsiella pneumoniae	12	0.46	26
Pseudomonas aeruginosa	-	-	25
Staphylococcus aureus	16	0.84	19
Bacillus cereus	-	-	29
Enterococcus faecalis	15	0.79	18

ZI-Zone of Inhibition AI–Activity Index mm–Millimeter

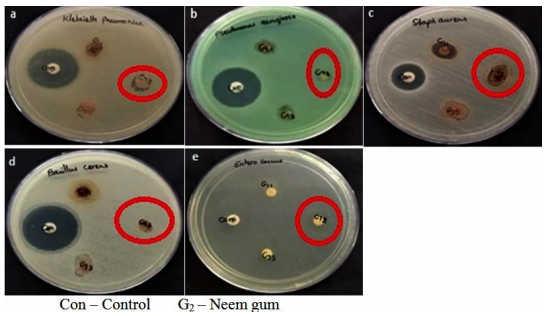

Fig. 8. AIG's Antibacterial activity (G2) against a) *Klebsiella pneumoniae*, b) *Pseudomonas aeruginosa*, c) *Staphylococcus aureus*, d) *Bacillus cereus*, e) *Enterococcus faecalis*

CONCLUSION

When secondary metabolites were tested, they found carbohydrates, alkaloids, phenols, phytosterols, Xanthoproteins, carboxylic acids, saponins, and tannins in neem gum. The functional biomolecules included in the gum were examined using Fourier transform infrared spectroscopy (FTIR). GCMS analysis shows the presence of important compounds like 4-Butyl-1-thia-cyclohexane, Z,Z-3,13-Octadecedien-1-ol, Estra-1,3,5(10)-trien-17-one, 3-methoxy-, 17-methoxime, carboxylic acid, cis-non-3-enyl ethyl ester, and 2,6-Lutidine 3,5-dichloro-4-dodecylthiol. The gum had antibacterial action against *Gram-positive* microorganisms *Staphylococcus aureus* (84%) and *Enterococcus faecalis* (79%). In accordance with the discoveries, this exploration will bring about the creation of an important atom that will be used to grow new, unique, and demanding antibacterial meds of normal beginning.

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Conflict of Interest

There are no irreconcilable situations pronounced by the creators.

REFERENCES

1. Arumugam.; P. Agullo.; T. Boopalan.; S. Nandy.; R. Lopez.; C. Gutierrez.; L. Rajkumar Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis Cancer Biology and Therapy., **2014**, *15*(1), 26-34, 10.4161/cbt.26604.
2. A.A. Oyagbemi.; O.A. Adejumobi.; E.V. Orherhe.; A.S. Amid.; A.A. Adedapo.; M.A. Yakuu Preconditioning with *Azadirachta indica* ameliorates cardiorenal dysfunction through reduction in oxidative stress and extracellular signal regulated protein kinase signalling *Journal of Ayurveda and Integrative Medicine.*, **2016**, *7*(4), 209 217, 10.1016/j. jaim.2016.08.006.
3. M. Patel.; K.C.N. Venkata.; P. Bhattacharyya.; G. Sethi.; A. Bishayee Potential of neem (*Azadirachta indica L.*) *for prevention and treatment of oncologic diseases Seminars in Cancer Biology.*, **2016**, *40–41*, 100-115, 10.1016/j.semcancer.2016.03.002.
4. R. Rupani.; A. Chavez Medicinal plants with traditional use: *Ethnobotany in the Indian subcontinent Clinics in Dermatology.*, **2018**, 36 (3), 306-309, 10.1016/j.clindermatol. 2018.03.005.
5. O. Verma.; S. Ponan.; A. Kamin Phytochemical screening of Ocimum sanctum (Tulsi), Azadirachta indica (Neem) and Phyllanthus emblica (Amla) *International Journal of Current Microbiology and Applied Sciences*, **2019**, *8*(4), 682-688.

6. L. Heyman.; Y. Houri-Haddad.; S.N. Heyman.; I. Ginsburg.; Y. Gleitman.; O. Feuerstein Combined antioxidant effects of Neem extract, bacteria, red blood cells and Lysozyme: *Possible relation to periodontal disease BMC Complementary and Alternative Medicine*., **2017**, *17*(1), 399, 10.1186/s12906-017-1900-3.
7. B. N. Joshi.; M. Bhat.; S.K. Kothiwale.; A.R. Tirmale.; S.Y. Bhargava Antidiabetic properties of azardiracta indica and bougainvillea spectabilis: *In vivo studies in murine diabetes model Evidence-Based Complementary and Alternative Medicine*., **2011**, *2010*, 1-10, 10.1093/ecam/nep033.
8. S. Saleem.; G. Muhammad.; M.A. Hussain.; S.N.A. Bukhari A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of *Azadirachta indica* Phytotherapy Research., **2018**, *32*(7), 1241-1272, 10.1002/ptr.6076.
9. M.A. Alzohairy Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment Evidence-Based Complementary and Alternative Medicine, **2016,** *2016*, 10. 1155/2016/7382506.
10. R. Al Akeel, A. Mateen, K. Janardhan, V.C. Gupta Analysis of anti-bacterial and anti oxidative activity of *Azadirachta indica* bark using various solvents extracts Saudi *Journal of Biological Sciences*., **2017**, *24*(1), 11-14, 10.1016/j.sjbs.2015.08.006.
11. W.N. Ghonmode.; O.D. Balsaraf.; V.H. Tambe.; K.P. Saujanya, A.K. Patil.; D.D. Kakde Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. feacalis*– An *In vitro* study *Journal of International Oral Health : JIOH*., **2013**, *5* (6), 61-66.
12. M.B. Yerima.; S.M. Jodi.; K. Oyinbo.; H.M. Maishanu.; A.A. Farouq.; A.U. Junaidu A.L. Shinkafi Effect of neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth Nigerian *Journal of Basic and Applied Science*., **2012**, *20*(1), 64-67.
13. M. Naik.; D. Agrawal.; R. Behera.; A. Bhattacharya.; S. Dehury.; S. Kumar Study of anti-inflammatory effect of neem seed oil (*Azadirachta indica*) on infected albino rats *Journal of Health Research and Reviews*., **2014**, *1*(3), 66, 10.4103/2394-2010.153880.
14. M. Schumacher.; C. Cerella.; S. Reuter.; M. Dicato.; M. Diederich Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-κB pathway Genes and Nutrition., **2011**, *6*(2), 149-160, 10.1007/ s12263-010-0194-6.
15. A.B. Shori.; A.S. Baba Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes and hypertension by *Azadirachta indica-yogurt Journal of Saudi Chemical Society*., **2013**, *17* (3), 295-301,10.1016/j. jscs.2011.04.006.
16. D.G. Soares.; A.M. Godin.; R.R. Menezes.; R.D. Nogueira.; A.M.S. Brito.; I.S.F. Melo.; R.R. *MachaoAnti-inflammatory and antinociceptive activities of Azadirachtin in mice Planta Medica*., **2014**, *80* (8–9), 630-636, 10.1055/s-0034-1368507.
17. G.H. Kumar.; R. Vidya Priyadarsini.; G. Vinothini.; P. Vidjaya Letchoumy.; S. Nagini *The neem limonoids azadirachtin and nimbolide inhibit cell proliferation and induce apoptosis in an animal model of oral oncogenesis Investigational New Drugs*., **2010**, *28*(4), 392-401,
18. S. Tapanelli.; G. Chianese.; L. Lucantoni, R.S. Yerbanga.; A. Habluetzel.; O. Taglialatela-Scafati Transmission blocking effects of neem (*Azadirachta indica*) seed kernel limonoids on Plasmodium berghei early sporogonic development Fitoterapia., **2016**, *114* , 122-126, 10.1016/j.fitote.2016.09.008
19. J. Zhu.; X. Lu.; X. Fan.; R. Wu.; H. Diao.; R. Yu.; J. Zi,; *A new cytotoxic salannin-class limonoid alkaloid from seeds of Azadirachta indica A. Juss Chinese Chemical Letters*., **2017**, *29*(8) 17-19, 10.1016/j.cclet.2017.11.042.
20. G. Shilpa.; J. Renjitha.; R. Saranga.; F.K. Sajin.; M.S. Nair.; B. Joy.; S. Priya *Epoxyazadiradione purified from the Azadirachta indica seed induced mitochondrial apoptosis and inhibition of NFκB nuclear translocation in human cervical cancer cells Phytotherapy Research*., **2017**, *31*(12), 1892-1902, 10.1002/ptr.5932

21. P. Manikandan.; P.V. Letchoumy.; M. Gopalakrishnan.; S. *Nagini Evaluation of Azadirachta indica leaf fractions for in vitro antioxidant potential and in vivo modulation of biomarkers of chemoprevention in the hamster buccal pouch carcinogenesis model Food and Chemical Toxicology*., **2008**, *46*(7) 2332-2343, 10.1016/j.fct.2008.03.013.
22. F. Hao.; S. Kumar.; N. Yadav.; D. *Chandra Neem components as potential agents for cancer prevention and treatment Biochimica et Biophysica Acta–Reviews on Cancer*., **2014**, *1846*(1), 247-257.
23. P. Elumalai.; D.N. Gunadharini.; K. Senthilkumar.; S. Banudevi.; R. Arunkumar.; C.S. Benson.; *J. Arunakaran Ethanolic neem (Azadirachta indica A. Juss) leaf extract induces apoptosis and inhibits the IGF signaling pathway in breast cancer cell lines Biomedicine and Preventive Nutrition*., **2012**, *2*(1), 59-68.
24. C. D. Mathers.; D. *Loncar Projections of global mortality and burden of disease from 2002 to 2030 PLoS Medicine*., **2006**, *3*(11), 2011-2030.
25. J. Upreti.; S. Ali.; S.F. *Basir Effect of lower doses of vanadate in combination with azadirachta indica leaf extract on hepatic and renal antioxidant enzymes in streptozotocin-induced diabetic rats Biological Trace Element Research*., **2013**, *156*(1), 202-209.
26. R.R. Ghatule.; G. Shalini.; M.K. Gautam.; A. Singh.; V.K. Joshi.; R.K. Goel Effect of Azadirachta indica leaves extract on acetic acid-induced colitis in rats: Role of antioxidants, free radicals and myeloperoxidase Asian Pacific *Journal of Tropical Disease*., **2012**, *2*(SUPPL2), S651-S657.
27. G. McCalla.; O. Prashad.; P. Brown.; M. *Gardner Beta cell regenerating potential of Azadirachta indica (Neem) extract in diabetic rats West Indian Medical Journal*., **2015**, *65* (1), 13-17.
28. B. Khillare.; T.G. *Shrivastav Spermicidal activity of Azadirachta indica (neem) leaf extract Contraception*., **2003**, *68*(3), 225-229, 10.1016/S0010-7824(03)00165-3.