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 ailments1, Neem (Azadirachta indica), a Meliaceae plant found in India, Pakistan, Bangladesh, and Nepal, has therapeutic implications in illness cure and formulation2. Although many chemicals are extracted from all parts of the Azadirachta plant, some chemicals extracted are tested for their pharmaceutical activities2. Quercetin and ß-sitosterol, two polyphenolic flavonoids extracted from fresh neem leaves, have antifungal and antibacterial properties3. The oil from Neem seed kernels contains a bitter crude component called Nimbidin have many biological actions4. 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 rats5,6. 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\textsuperscript{7,8}. Since 1959, nimbidin and nimbin have been shown to have spermicidal action in rats and humans\textsuperscript{9}. Nimbolide inhibits the growth of Plasmodium falciparum, indicating antimalarial activity\textsuperscript{10}. Nimbolide acts as an antimicrobial agent and shows high activity against Staphylococcus aureus and Staphylococcus coagulase\textsuperscript{11}. Azadirachta seed oil contains a compound named Gedunin that has good antimicrobial and antimalarial properties\textsuperscript{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\textsuperscript{13}. Mahmoodin, a deoxygedunin derived from seed oil, was found to have moderate antibacterial activity against various human pathogenic bacteria strains\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{21}. Moreover, it was found to contain natural unsaturated fats\textsuperscript{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)\textsuperscript{23}, paper (cement and paper fortifying)\textsuperscript{24}, drugs (sterile creams, tablet folio, and coater)\textsuperscript{25}, materials (texture coloring and printing)\textsuperscript{24}, and food (balancing out specialist, gels, and thickening specialists)\textsuperscript{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\textsuperscript{-1}–400 cm\textsuperscript{-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 dioxane), 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.

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

**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.

| 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.

**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-thiacyclohexane, Z, Z-3,13-Octadecadien-1-old, Estr-1,3,5(10)-trien-17-one, 3-methoxy-17-methoxime, Adipic corrosive, cis-non-3-ethyl 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_18S     | 158      | 22.85  |
| 2    | 38.60          | 6-Bromohexanoic acid, isopropyl ester  | C_9H_17BrO_2 | 236      | 22.53  |
| 3    | 38.747         | Adipic acid, cis-non-3-enyl ethyl ester| C_7H_14NO_2  | 298      | 22.23  |
| 4    | 38.765         | Bicyclo[4.1.0]heptan-2-OL              | C_9H_12O     | 154      | 19.73  |
| 5    | 38.855         | Z,Z-3,13-Octadecadien-1-ol             | C_18H_34O     | 266      | 16.41  |
| 6    | 38.860         | Ethyl 9-hexadecenoate                  | C_9H_18O     | 282      | 16.62  |
| 7    | 38.985         | 2,6-Lutidine 3,5-dichloro-4-dodecylthiol| C_19H_31Cl_2NS | 375      | 23.05  |
| 8    | 38.995         | Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl] | C_9H_17NO_2 | 207      | 20.05  |
| 9    | 39.015         | Estra-1,3,5(10)-trien-17-one, 3-methoxy-,17-methoxime | C_20H_27NO_2 | 313      | 15.46  |
| 10   | 39.025         | 3-Methoxy-1,3,5(10)-estratriene-17-methoxime | C_20H_27NO_2 | 313      | 19.43  |

**Figure 2.** AIG’s GC-MS total Chromatograph (G2)

**Figure 3.** 4-butyl-1-thia-cyclohexane EI mass spectrum

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

**Figure 5.** Z,Z-3,13-Octadecadien-1-ol EI mass spectrum

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

**Figure 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_18S 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_7H_14NO_2 is liable for the top at m/z 128 with R.T. 38.745. The EI mass range of Z, Z-3,13-Octadecadien-1-ol 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_18H_34O. 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 Control (mm) | Control (Nystatin mm) |
|-----------------------------|-------------------------------------|-----------------------|
| Klebsiella pneumoniae       | 12                                  | 0.46                   |
| Pseudomonas aeruginosa      | -                                   | -                     |
| Staphylococcus aureus       | 16                                  | 0.84                   |
| Bacillus cereus             | -                                   | -                     |
| Enterococcus faecalis       | 15                                  | 0.79                   |

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

**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.

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