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# ANTIOXIDANT ACTIVITY, MOSQUITOCIDAL ACTIVITY, ANTIBACTERIAL ACTIVITY OF SOLVENT EXTRACT AND SYNTHESIZED NANOPARTICLES OF *CLAUSENA EXCAVATA* (BURM.) F. (RUTACEAE) AGAINST SELECTED MOSQUITOES AND PATHOGENIC BACTERIA

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## Abstract

*In the present investigation, Clausena excavata an indegeous plant was sequentially extracted with hexane, ethyl acetate and methanol and their bio efficacy in terms of Antioxidant activity, mosquitocidal activity and antibacterial activity was tested. Among the three-extract methanol extract of C. excavata displayed significant antioxidant, larvicidal, ovicidal, pupicidal activities against Aedes aegypti and Culex quinquefasciatus. Similarly, methanol extract also showed pronounced antibacterial activity against the selected human pathogenic bacteria. Thus, the methanol extract of C. excavata was used to synthesize silver mediated nanoparticles and its (C.*

*excavata AgNPs) bio efficacy was also tested with the selected vector mosquitoes and selected human pathogenic bacteria. This present investigation envisages the possible utilization of C. excavata as a potent candidate as green pesticides in the near future.*

**Keywords**

*Clausena Excavata, Ae. Aegypti, C. Quinquefasciatus,* Antioxidant Activity, Antibacterial Activity, Green Pesticides

# 1. Introduction

## 1.1 Antioxidant Activities of Plants

Antioxidant molecules from plant origin can save the cell from free radicles leading to chain reactions that may harm the cells. A number of researches have been documented to correlate amount of phenolic compounds in plants and antioxidant activity (Alali *et al.,* 2007; Gan *et al.,* 2010 and Spiridon *et al.,* 2011; Bild *et al*., 2013; Rahal *et al*., 2014; Lushchak, 2014; Bosha, 2015). In the present study *Clausena excavata* a medicinal plant widely distributed in Southeast Asia is used in folklore medicine for the treatment of several illnesses. Recent studies showed that the plant also possessed immuno-modulatory, anti-inflammatory, antivirus, anticancer (Sharif *et al.* 2011), antioxidant (Guntupalli *et al.,* 2012), and antifungal (Kumar *et al.,* 2012) activities. *C. excavata* has been reported to exhibit one of the highest beneficial biological activities among *Clausena* genus, furthermore, it also exhibits strong mosquitocidal activity against the important vector mosquitoes (Mathivanan *et al*., 2014).

Arthropods are awfully perilous vectors of pathogens and parasites (Bonizzoni *et al.,* 2013; Mehlhorn, 2015; Benelli *et al*., 2016). Among them, mosquitoes signify a huge threat for millions of people all over the world (Benelli and Mehlhorn, 2016; Pastula *et al*., 2016; Saxena *et al*., 2016). Meanwhile previous few years, dengue fever has become the most important public-health concern in tropical and subtropical regions of the world (WHO, 2015). It has been estimated that, 3900 million individuals from 128 countries, are at the risk of dengue Union Health Ministry reveal a massive increase in dengue infections every year in India, (National Vector Borne Disease Control Programme, 2015).

The acquaintance and use of a wide spectrum of medicinal plants have been documented to combat various infectious diseases impeding human life and activity (Pfaller and Diekema, 2012). The unfettered use of antimicrobial agents and poor hygienic conditions severely affects

human beings (Cavallo *et al*., 2007; Eber *et al*., 2010; Rao *et al*., 2011; Kwak *et al*., 2012; Ko *et al.,* 2013; Dag *et al*., 2014 and Mousavi *et al*., 2015; Najari and Alimohammadi, 2015; Paproski *et al*., 2015). Several researchers have been carried out to study the antimicrobial activity of different plant extracts (Rajeshkumar, 2014 and 2015; Chandrika and Chellaram. 2015; Gladis Raja Malar and Chellaram. 2016).

The applications of nanoparticles and nanomaterials are having immense role due to their nano size and morphology. Formidable development in these emerging technologies opened an pragmatic fields and novel necessities (Balagurunathan *et al.,* 2011). Synthesis of silver nanoparticles from native plants is gaining more attention, because of their fast, eco-friendly, non-pathogenic nature and their binding ability was well described (Nabikhan *et al.,* 2010; Gopinatha *et al.,* 2012; Ramya and Subapriya, 2012; Veeraputhiran, 2013; Geetha *et al.,* 2014; Kulkarni and Muddapur *et al.,* 2014; Kumar *et al.,* 2014; Nakkala *et al.,* 2014; Suna *et al.,* 2014; Mariselvam *et al.,* 2014; Ashok kumar *et al.,*2015; Sadeghi and Gholamhoseinpoor *et al.,* 2015; Nasrollahzadeh *et al*., 2016).

## 2. Materials and Methods

### 2.1 Processing of Plant Extracts

The fresh leaves of *Clausena excavata* (Rutaceae) the plant material was collected from Yercaud hills, Salem District of Tamilnadu and its geographical coordinates are 11° 46' 0" North, 78° 14' 0" East. The plant materials were shade dried in the laboratory; powdered using electric blender and the powder was sequentially extracted with hexane, ethyl acetate and methanol in Soxhelt apparatus. The crude extracts were individually stored at 4 $^0$C. The diluted concentration was used for subsequent experiments (Plate 4.1 & 4.2).

### 2.2 DPPH Assay

The antioxidant activity of different extracts was determined by using DPPH assay. The decrease in the absorption of DPPH solution at 517nm after the addition of antioxidant was measured in a cuvette containing 2.960µl of 0.1mM methanolic DPPH solution; 20, 40, 60, 80 and 100µg/mL of crude extracts. After 20 minutes, the ability of the plant extract to scavenge DPPH radical was calculated by the following equation $\%\text{RSA}\frac{\text{Ac}-\text{As}}{\text{Ac}}\text{X}100$

Where Ac=Absorbance of control; As=Absorbance of sample

*Abs*. control = Absorbance of DPPH radical + methanol *Abs*. sample = absorbance of DPPH radical + plant extract.

**2.3 FRAP Assay**

FRAP assay of the selected plant extracts were performed by adapting the procedure prescribed by Iris *et al*. (1996).

**2.4 Total Flavonoids and Total Phenolic Content**

Total flavonoids and phenolic content was estimated using the method prescribed by Harborne *et al*. (1973).

**2.5 Raring of Mosquitoes in Laboratory**

Eggs and larvae of *A. aegypti and C. quinquefasciatus* were continuously reared by following the protocol prescribed by (Elumalai and Kasinathan, 2016)

**Larvicidal Bioassay:** Larvicidal and Ovicidal activity of the extract was determined by following the standard procedure (WHO, 2005).

$$\%\text{Ovicidal Activity} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs treated}} \text{ X } 100$$

**2.7 Biosynthesis and Characterization of Silver Nanoparticles:** The method prescribed by Iravani et al. (2014) was adapted with little modifications.

**2.8 Antimicrobial Assay – Disc Diffusion Methods**

*In vitro* antimicrobial assay was carried out for the crude extracts of *C. excavata* as per the method prescribed by Iwu et al. (1999) against 8 bacterial strains, which includes 4 Gram-positive bacteria (*Bacillus subtilis* (MTCC:441), *Micrococcus luteus* (MTCC:1538), *Staphylococcus aureus* (MTCC:96) and *Streptococcus mutans* (MTCC:497) and 4 Gram-negative bacteria *Escherichia coli* (MTCC:443), *Klebsiella pneumoniae* (MTCC:109), *Proteus vulgaris* (MTCC:426) and *Schigella flexneri* (MTCC:1457).

## 3. Results

C.excavata crude extracts showed the presence of several phytochemical groups and are shown in table 1. In the same way, the total phenolic and total flavonoid contents of C. excavata are shown in Table 2. Antioxidant potential of C. excavata extracts were tested with DPPH assay (Figures 1-3), and FRAP (Figure 4). Furthermore, the mosquitocidal activities of C. excavata crude extracts for their larvicidal activity against the fourth instar larvae of Ae. aegypti and C.

quinquefasciatus revealed that methanol extract induced remarkable larval mortality against Ae. aegypti than C. quinquefasciatus (Table 3 &3a). Similarly, the data pertaining to ovicidal activity are shown in Table 4 and 5. In the same way, the synthesized silver nanoparticles from the methanolic leaf extract of C. excatava were tested for their larvicidal and ovicidal activity and the data pertaining to the experiments are shown in tables 6 and 7. Antibacterial activity of extracts of C. excavata were also tested in the present investigation against the selected gram ositive bacteria and gram negative bacteria clearly revealed that among the gram-positive bacteria, Bacillus subtilis was found more susceptible (32mm zone of inhibition). Besides a 32mm zone of inhibition was also noted in the gram-negative bacteria Proteus vulgaris (Table 8). Table 9 shows the MIC values of different solvent extract of C. excavata. The characterization of SNPs of C. excavata was analyzed with various spectral analysis such as SEM (Figure 5 & Plate 1) XRD (X ray Diffraction of AGNPS of C. excavata are shown in Figure 6 and its Zeta potential of AGNPS was analyzed and results are shown in Figure 7.

**Table 1:** *Qualitative Analysis of Phytochemical in Different Solvent of Clausena Excavata Leaf*

| **Phytochemical Groups** | **Extracts Tested** | | |
|---|---|---|---|
| | **Hexane Extract** | **Ethyl acetate Extract** | **Methanol Extract** |
| Alakaloids | + | + | + |
| Anthoquions | - | - | - |
| Coumarins | + | + | + |
| Flavonoids | + | - | + |
| Phenols | + | + | + |
| Resins | - | + | - |
| Saponins | + | + | + |
| Steroids | + | - | - |
| Tannins | + | + | + |
| Terpenoids | - | + | + |

(+)-Presence, (-) - Absence

**Table 2:** *Estimation of Total Flavonoids and Phenolics of Different Extracts of Clausena Excavata*

| Contents | Extracts analyzed | | |
|---|---|---|---|
| | **Hexane** | **Ethyl acetate** | **Methanol** |
| Total Phenolic* | 7.24 ± 0.22[a] | 10.16 ± 0.27[a] | 11.42 ± 0.61[a] |
| Total Flavonoids** | 8.54 ± 0.84[b] | 37.12 ± 0.63[b] | 47.28 ± 0.32[b] |

Values represent mean ± S.D of three replications. Values holding different alphabet in the column are differ significantly at $p<0.05$% (DMRT). *µg gallic acid equivalent (GAE)/g DW. ** µg rutin equivalent/g DW.

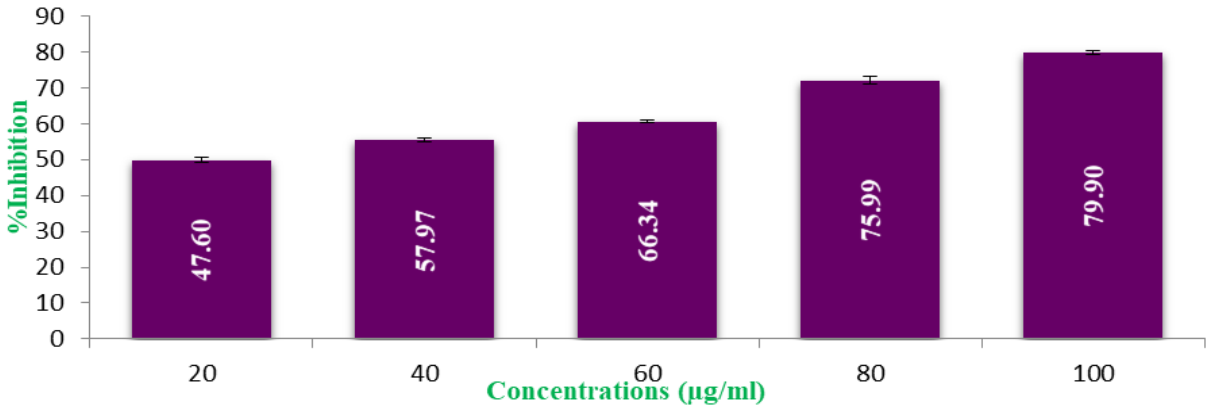


**Figure 1:** *Antioxidant Activity (2, 2-diphenyl-1-picrylhydrazyl assay) of Hexane Extract of Clausena Excavata Leaf*

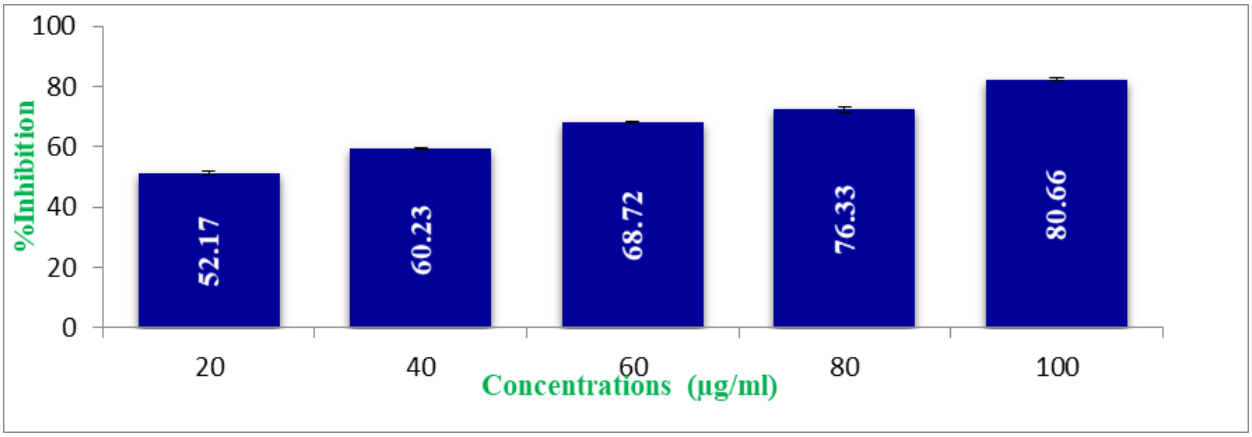


**Figure 2:** *Antioxidant Activity (2, 2-diphenyl-1-picrylhydrazyl assay) of Ethyl Acetate Extract of Clausena Excavata Leaf*

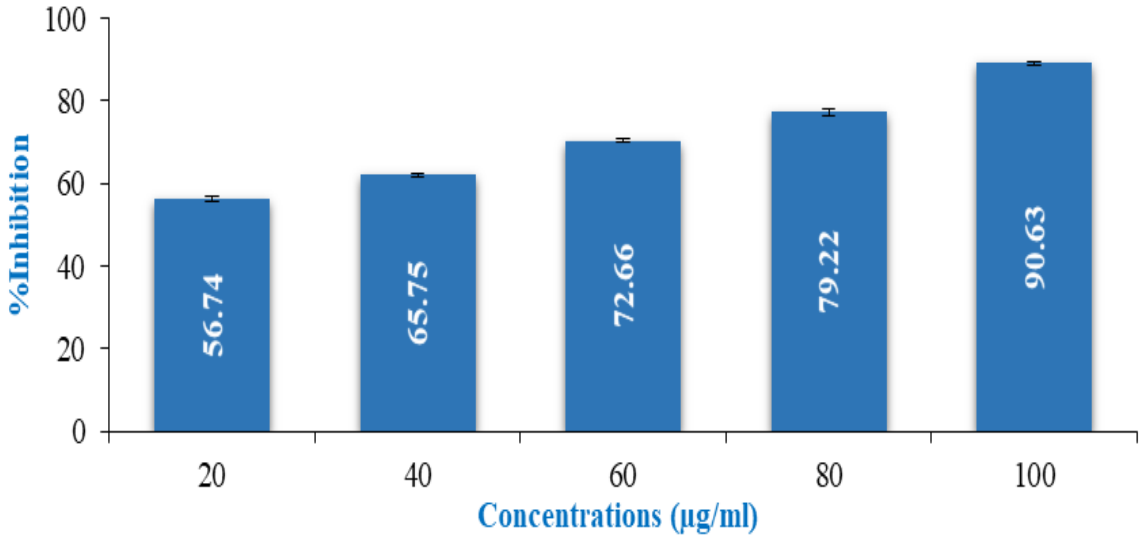


**Figure 3:** *Antioxidant activity (2, 2-diphenyl-1-picrylhydrazyl assay) of methanol extract of Clausena excavata leaf*

**Antioxidant activity (FRAP) of different solvent extracts of *C. excavata***

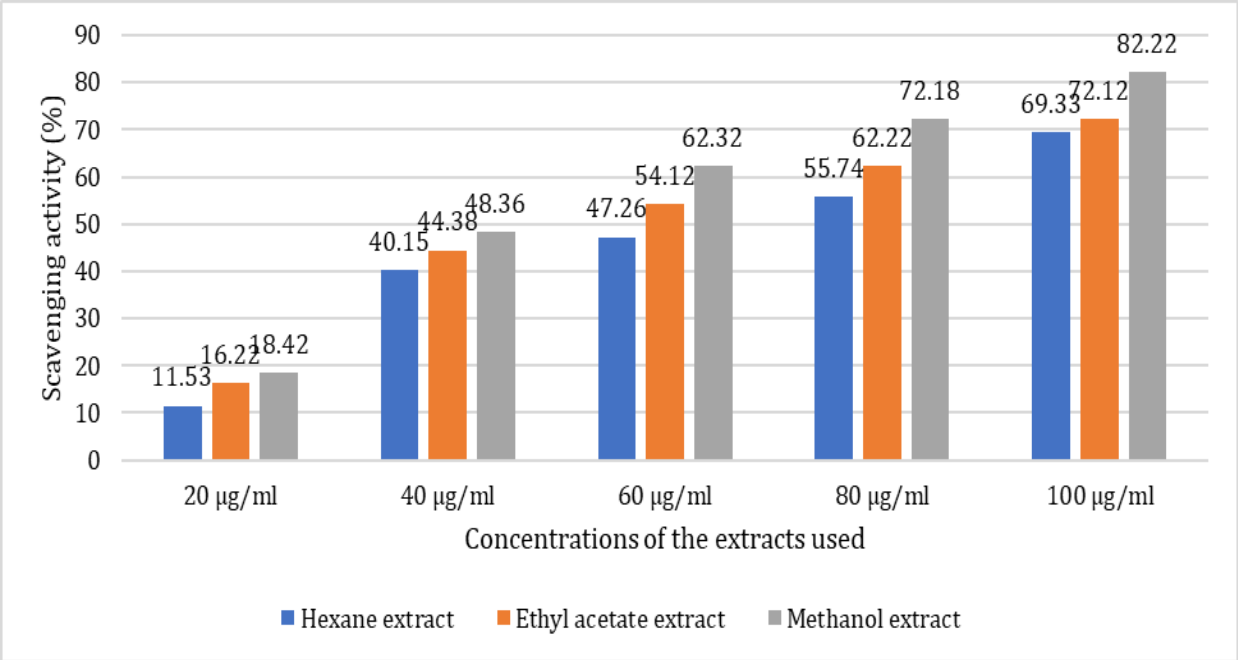


**Figure 4:** *Antioxidant Activity (FRAP assay) of Different Solvent Extract of Clausena Excavata Leaf*

Values represent mean of five replications. FRAP- Ferric Reducing Antioxidant Power.

**Table 3:** *Larvicidal Activities of Hexane, Ethyl Acetate and Methanol Extract of C. Excavata Tested against the Aedes Aegypti and Culex Quinquefasciatus*

| Extracts tested | Concentrations | *Aedes aegypti* | *Culex quinquefasciatus* |
|---|---|---|---|
| | | Mortality (%) | |
| Hexane extract | 31.25 | 17.2 ± 4.1[a] | 18.6 ± 5.9[a] |
| | 62.5 | 33.8 ± 5.7[b] | 34.6 ± 4.1[b] |
| | 125 | 67.6 ± 2.0[c] | 66.8 ± 7.3[c] |
| | 250 | 94.4 ± 4.7[d] | 90.2 ± 7.4[d] |
| Ethyl acetate extract | 31.25 | 30.4 ± 10.8[a] | 1.2 ± 0.4[a] |
| | 62.5 | 64.0 ± 9.0[b] | 26.6 ± 3.7[b] |
| | 125 | 75.4 ± 8.8[c] | 40.8 ± 13.0[c] |
| | 250 | 100.0±0.0[d] | 80.4 ± 3.5[d] |
| Methanol extract | 31.25 | 36.2±6.1[a] | 30.6±4.6[a] |
| | 62.5 | 69.2±6.6[b] | 63.4±6.1[b] |
| | 125 | 100.0±0.0[c] | 94.8±7.0[c] |
| | 250 | 100.0±0.0[d] | 100.0±0.0[d] |

The value represents mean ±S. D. of five replications. *mortality of the larvae observed after 24h of the exposureperiod, WHO (2005).

**Table 3A:** *Determined Lethal Concentrations of Different Solvent Extract of C. Excavata Tested against the Aedes Aegypti and Culex Quinquefasciatus*

| Mosquito species | **$LC_{50}$ (ppm)** (LCL – UCL) | **$LC_{90}$ (ppm)** (LCL – UCL) | **$\chi^2$ value** |
|---|---|---|---|
| **Hexane extract** | | | |
| *Aedes aegypti* | 95.40<br>83.751-07.39 | 198.27<br>178.20- 226.19 | 4.110 |
| *Culex quinquefasciatus* | 103.49<br>(11.16 - 206.49) | 227.47<br>(155.19-780.50) | 7.562 |
| Ethyl acetate extract | | | |
| *Aedes aegypti* | 57.92<br>(74.28- 67.30) | 136.67<br>(120.79-161.13) | 0.286 |

| | | | |
|---|---|---|---|
| *Culex quinquefasciatus* | 72.82 (62.60-82.83) | 157.13 (140.04-182.11) | 4.689 |
| Methanol extract | | | |
| *Aedes aegypti* | 42.05 (34.55-48.25) | 87.39 (77.67-103.04) | 2.457 |
| *Culex quinquefasciatus* | 49.81 (41.14-57.34) | 108.79 (97.24-126.03) | 0.929 |

$LC_{50}$=Lethal Concentration brings out 50% Mortality and $LC_{90}$ = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ (MANOVA; LSD -Duncan Multiple Range Test).

**Table 4:** *Ovicidal Activity of Different Extracts of Clausena Excavata Tested against the Eggs of Aedes Aegypti*

| **Name of the species** | **Ovicidal activity (%)** | | | |
|---|---|---|---|---|
| | **Concentrations tested (ppm)** | | | |
| | **31.25** | **62.5** | **125** | **250** |
| Hexane | $13.0 \pm 3.8^{a}$ | $52.0 \pm 2.3^{a}$ | $63.6 \pm 6.5^{a}$ | $71.8 \pm 2.8^{a}$ |
| Ethyl acetate | $34.4 \pm 6.0^{b}$ | $61.2 \pm 9.6^{b}$ | $74.4 \pm 3.8^{b}$ | $88.2 \pm 3.3^{b}$ |
| Methanol | $47.4 \pm 5.9^{c}$ | $65.8 \pm 4.4^{c}$ | $82.6 \pm 3.6^{c}$ | $94.0 \pm 2.5^{c}$ |
| Neem azal | $100 \pm 0.00^{d}$ | $100 \pm 0.00^{d}$ | $100 \pm 0.00^{d}$ | $100 \pm 0.00^{d}$ |

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at $p<0.05$; LSD-Duncan Multiple Range Test). Eggs in the control groups were sprayed with no phytochemicals (Su and Mulla, 1998 and Abbott 1925).

**Table 5:** *Ovicidal Activity of different Extracts of Clausena Excavata Tested against the Eggs of Culex Quinquefasciatus*

| **Name of the species** | **Ovicidal activity (%)** | | | |
|---|---|---|---|---|
| | **Concentrations tested (ppm)** | | | |
| | **100** | **200** | **300** | **400** |
| Hexane | $16.2 \pm 3.1^{a}$ | $39.8 \pm 2.4^{a}$ | $61.4 \pm 3.8^{a}$ | $77.0 \pm 2.9^{a}$ |
| Ethyl acetate | $46.4 \pm 5.5^{b}$ | $70.0 \pm 3.3^{b}$ | $88.4 \pm 2.9^{b}$ | $92.8 \pm 2.2^{b}$ |
| Methanol | $53.4 \pm 3.9^{c}$ | $74.0 \pm 4.1^{c}$ | $85.2 \pm 12.7^{c}$ | $97.0 \pm 2.0^{c}$ |
| Neem azal | $100 \pm 0.00^{d}$ | $100 \pm 0.00^{d}$ | $100 \pm + 0.00^{d}$ | $100 \pm 0.00^{d}$ |

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at $p<0.05$;LSD -Duncan Multiple Range Test). Eggs in the control groups were sprayed with no phytochemicals (Su and Mulla, 1998 and Abbott 1925).

**Table 6:** *Larvicidal Activity of Clausena Excavata Coated with Silver Nanoparticles tested against the Larvae of Aedes Aegypti and Culex Quinquefasciatus*

| Concentrations | Larvicidal activity (%) | |
|---|---|---|
| | ***Aedes aegypti*** | ***Culex quinquefasciatus*** |
| 10µg/ml | $26.4 \pm 2.2^{a}$ | $49.1 \pm 2.9^{a}$ |
| 15 µg/ml | $62.8 \pm 8.1^{b}$ | $72.6 \pm 3.7^{b}$ |
| 20 µg/ml | $94.6 \pm 4.6^{c}$ | $100.0 \pm 0.0^{c}$ |
| AgNO3 (0.05 µg/ml) | $100 \pm 0.0^{d}$ | $100.0 \pm 0.0^{c}$ |

Value represents mean ± S.D. of five replications. *mortality of the larvae observed after 24h of exposure period, WHO (2005). Values with a different superscript alphabet are significantly different at $P < 0.05$ (MANOVA; LSD - Tukey's Test).

**Table 7:** *Ovicidal Activity of Clausena Excavata Coated with Silver Nanoparticles Tested against the eggs of Aedes aegypti and Culex Quinquefasciatus*

| Concentrations | Ovicidal activity (%) | |
|---|---|---|
| | ***Aedes aegypti*** | ***Culex quinquefasciatus*** |
| 10µg/ml | $44.6 \pm 4.3^{a}$ | $52.4 \pm 10.2^{a}$ |
| 15 µg/ml | $66.8 \pm 3.1^{b}$ | $86.6 \pm 4.3^{b}$ |
| 20 µg/ml | $92.8 \pm 1.6^{c}$ | $100.0 \pm 0.0^{c}$ |
| $AgNO_3$ ( 0.01 µg/ml) | $100.0 \pm 0.0^{d}$ | $100.0 \pm 0.0^{c}$ |

Value represents mean ± S.D. of five replications. *mortality of the legs observed after 24h of exposure period, WHO (2005). Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ (LSD - Duncan Multiple Range Test).

**Table 8:** *Antibacterial Activity of Different Solvent Extracts of Clausena Excavata leaf tested against Selected Human Pathogenic Bacteria*

| Microorganism tested | | Zone of inhibition(mm) diameter | | | |
|---|---|---|---|---|---|
| | | Organic solvents tested | | | |
| | | **PC*** | **HEX** | **EA** | **ME** |
| Gram positive | *Bacillus subtilis* | 11 | 0 | 20 | 32 |
| | *Micrococcus luteus* | 13 | 0 | 18 | 28 |
| | *Staphylococcus aureus* | 9 | 0 | 16 | 26 |

| | | | | | |
|---|---|---|---|---|---|
| | *Streptococcus mutans* | 0 | 0 | 0 | 0 |
| Gram negative | *Escherichia coli* | 0 | 0 | 15 | 25 |
| | *Klebsiella pneumonia* | 14 | 0 | 20 | 30 |
| | *Proteus vulgaris* | 17 | 0 | 14 | 32 |
| | *Schigella flexnari* | 10 | 7 | 22 | 27 |

**PC***= Positive Control *i.e.,* Cefalexin 10µg and Gentamycin 10µg for gram positive and gram negative bacteria respectively; **HEX** = Hexane extract; **EA**= Ethyl acetate extract; **ME** = Methanol extract.

**Table 9:** *Antibacterial Activity (Minimum Inhibitory Concentration) of Different Solvent Extracts of Clausena Excavata (leaf) against the Selected Human Pathogenic Bacteria*

| **Microorganism tested** | | **Minimum inhibitory concentration (µg/ml)** | | | |
|---|---|---|---|---|---|
| | | **Organic solvents tested** | | | |
| | | **PC*** | **HEX** | **EA** | **ME** |
| Gram positive | *Bacillus subtilis* | 3.16 | 56.21 | 40.36 | 96.4 |
| | *Micrococcus luteus* | 5.38 | 12.29 | 165.3 | 44.9 |
| | *Staphylococcus aureus* | 3.11 | 17.65 | 122.8 | 189.6 |
| | *Streptococcus mutans* | 4.76 | 22.89 | 52.14 | 46.7 |
| Gram negative | *Escherichia coli* | 8.87 | 86.14 | 23.47 | 125.1 |
| | *Klebsiella pneumonia* | 12.9 | 123.45 | 95.14 | 98.6 |
| | *Proteus vulgaris* | 16.4 | 44.35 | 24.77 | 117.8 |
| | *Schigella flexnari* | 7.55 | 17.8 | 69.28 | 157.2 |

**PC***= Positive Control *i.e.,* Cefalexin 10µg and Gentamycin 10µg for gram positive and gram negative bacteria respectively; **HEX** = Hexane extract; **EA**= Ethyl acetate extract; **ME** = Methanol extract

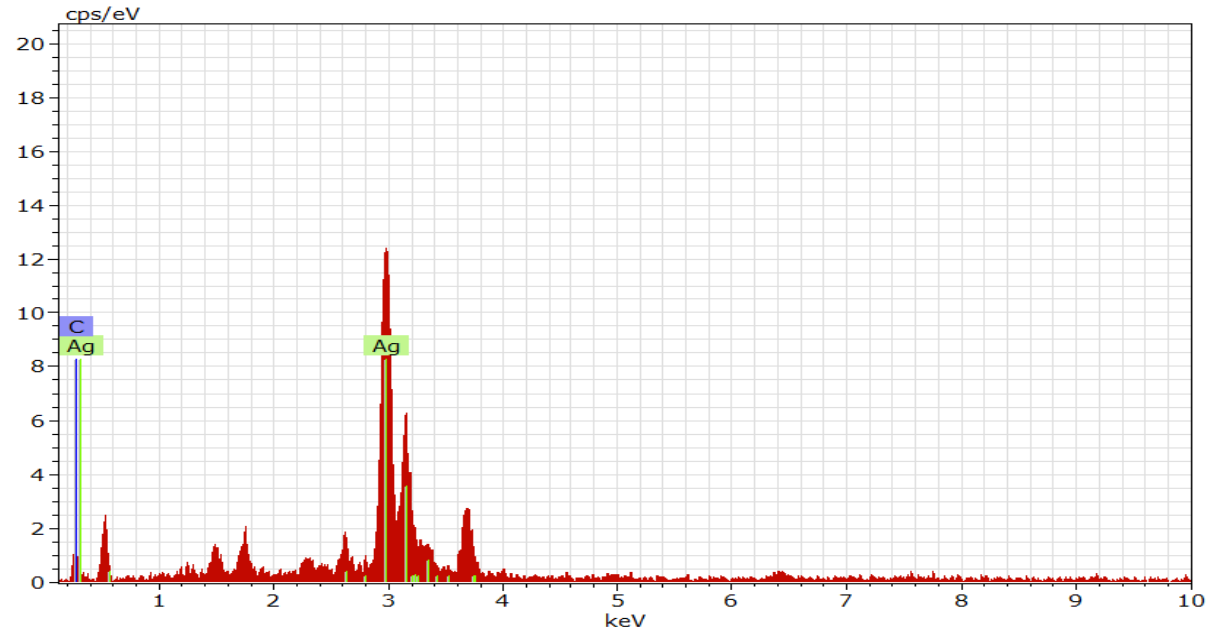


**Figure 5:** *EDAX of Synthesized Silver Nano Particle of Clausena Excavata*

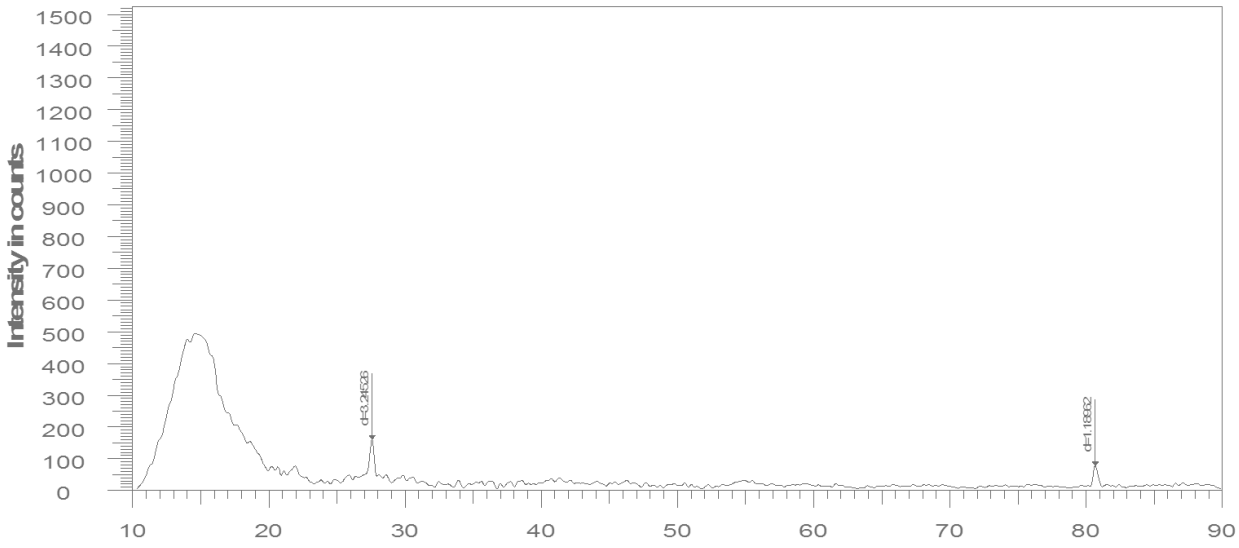


**Figure 6:** *XRD Pattern of Synthesized Silver Nanoparticle of Clausena Excavata*

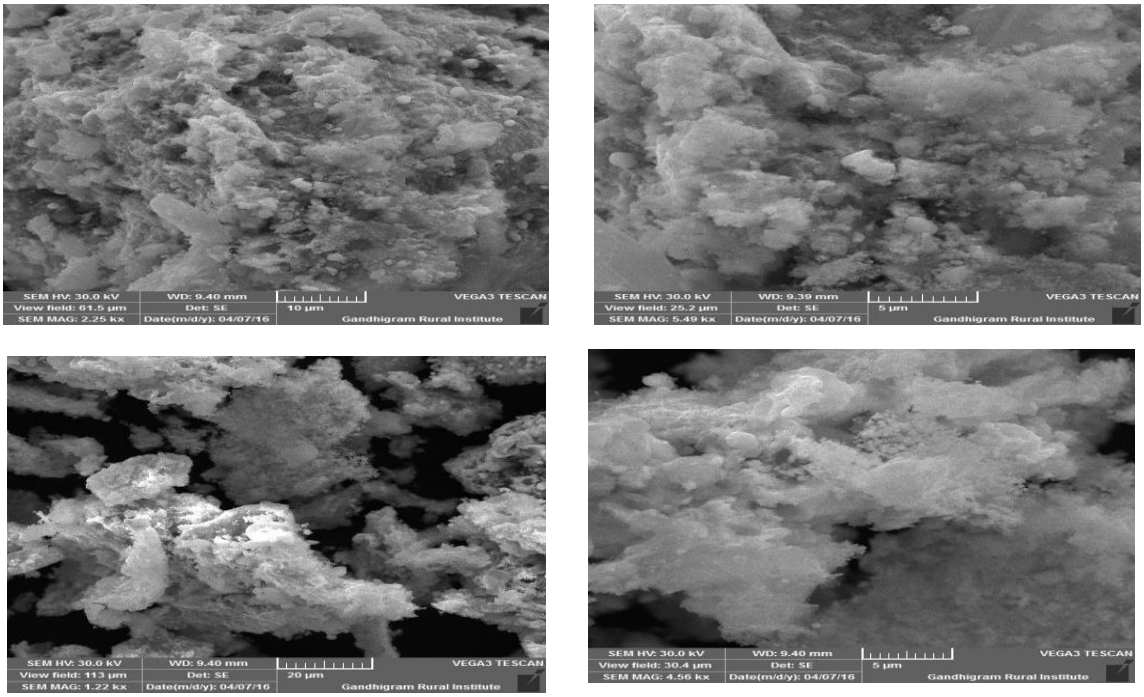


**Figure 7:** *Photograph showing Scanning Electron Micrograph (SEM) of synthesized silver nano particle of Clausena excavata*

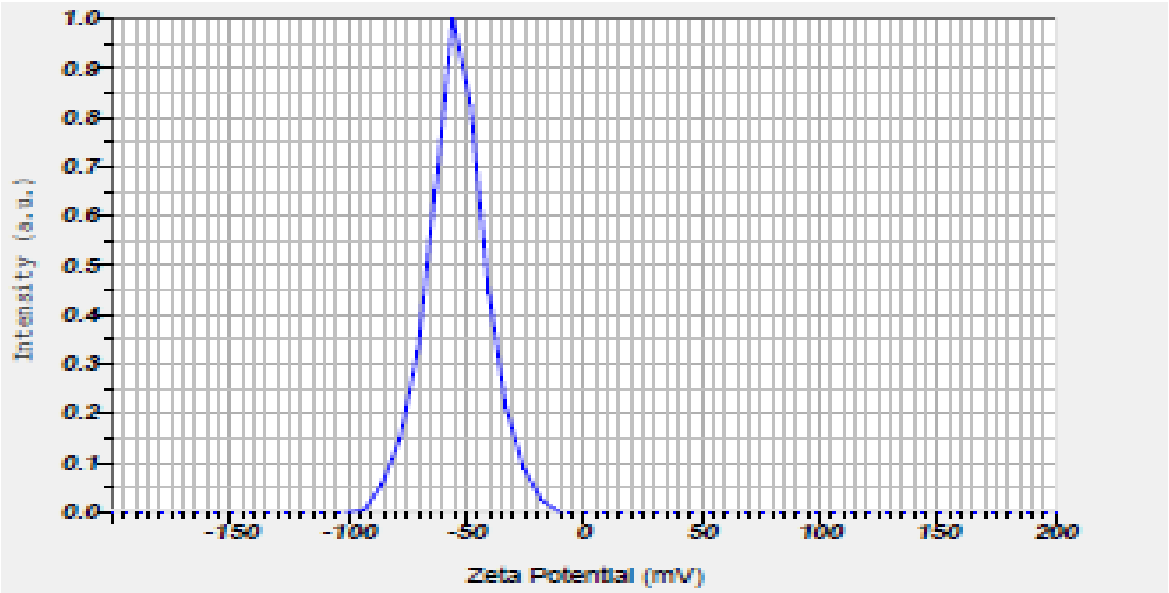


**Figure 8:** *ZETA Potential of Clausena Excavate SNP*

| | | |
|---|---|---|
| Measurement Type | : | Zeta Potential |
| Sample Name | : | HM1silver-z |
| Temperature of the Holder | : | 25.2 °C |
| Dispersion Medium Viscosity | : | 0.892 mPa·s |
| Conductivity | : | 0.433 mS/cm |
| Electrode Voltage | : | 3.3 V |

**Calculation Results**

| Peak No. | Zeta Potential | Electrophoretic Mobility |
|---|---|---|
| 1 | -54.0 mV | -0.000419 cm2/Vs |
| 2 | --- mV | --- cm2/Vs |
| 3 | --- mV | --- cm2/Vs |

| | | |
|---|---|---|
| Zeta Potential (Mean) | : | -54.0 mV |
| Electrophoretic Mobility Mean | : | -0.000419 $cm^2/Vs$ |

## 4. Discussion

In the present study methanol leaf extract of *Clausena excavata* showed increased DPPH $NO_2$ and FRAP activity. Likewise, the phytochemical present in the solvent extracts also inhibited the free radicals in antioxidant activity method. Presence of terpenoids have been confirmed in the phytochemical screening and it has been suggested that it may be responsible for antioxidant and antimicrobial properties (Singh and Singh, 2003). Presence of phenols in extract may elucidate its t bioactivities as tannins are known to possess potent antioxidants (Pereira *et al*., 2007). The present investigation the strong antioxidant activity was confirmed in methanol extract could be due to the strong occurrence of polyphenolic compound such as terpenoids and phenols. These findings provide scientific evidence to support old-style uses and indicate a promising potential for the development an antimicrobial and antioxidant drug from *C. excavata* plant.

In the present study the antioxidant activity of *C. excavata* extracts were analyzed with DPPH, FRAP assays to confirm its potentiality to be a antioxidant (Zengin *et al.,* 2011; Kumar *et al.,* 2014; Floegel *et al*., 2011; Mehta *et al.,* 2013). *C.excavata* (leaves) displayed compelling antioxidant activity and also the total phenolic and flavonoid contents in order to find possible sources for future novel antioxidants In this present study, crude extract of *C.excavata* was screened to regulate their active chemical constituents using conventional chemical tests (precipitation and color reagents) and antioxidant activities were determined using the DPPH radical scavenging method (Elumalai and Kasinathan, 2016).

This study reveals that the *C. excavata* has remarkable ovicidal and repellent properties against *Ae. aegypti* and *C. quinquefasciatus* mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants. In the last decades, there has been particular interest in the use of naturally abundant antimicrobial agents from plants. Antimicrobial agents are chemical compounds derived from herbs, shrubs and or whole plants. Basically, there are two ways to control or inhibit the growth of microorganisms, *i.e.* through physical or chemical agents, where choice is made on the basis of the situation. It has been well documented by the researchers that the plant provides a good platform for the control of various pathogenic bacteria (Iwu *et al*., 1999; Mohana and Raveesha, 2006; Parekh and Chanda, 2007; Aiyegoro *et al.*, 2008; Kalpana *et al*., 2013; Ashok *et al*., 2014). In those studies, different extracts obtained from various parts such as roots (Raj *et al*., 2011, leaves (Ashok *et al*., 2014, Kalpana *et al*., 2013), bark (Zaffer *et al*., 2014) and) has been studied for their antimicrobial activity against different types of microorganisms (Rajeshkumar 2014 and 2015; Kumar *et al* 2009; Amanpour *et al* 2015).

**Conclusion and Recommendations:** Despite the large variety of plants are listed in India, relatively few informations are available for their efficacy against the control of field pest, storage pests, vector mosquitoes, antioxidant and antibacterial activities. Based on studies undertaken in the present investigation, the methanol extract of *C. excavata* was found to be the most efficacious against the selected vectors and pathogenic bacteris. The results indicated that the selected plant will provide evidence for efficacy thereby generating confidence towards the development of a formulation towards the control of the organisms tested in the present research. There is always a general opinion that the crude extracts are often more efficacious than isolated fractions, but the present research elucidates newer approaches for standardization of isolated and identified phytochemical compounds. This is important for developing a formulation with uniform efficacy and wider acceptability using phytochemicals thereby serving as a prototype for modernization of traditional method of intensive vector control program, and also the traditional medicinal system for the prohibition of pathogenic bacterial infection.

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