

Antioxidant and Cytotoxic agent from *Acacia catechu* Stem Bark against MCF-7 Cell Line

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ABSTRACT

Anti-cancer agents can be found in a variety of natural products because of their chemical and biological activity. Free radicals are molecules with high instability which induced initiation and progression of tumor formation. In contrast, the compositions of antioxidant material act as defense activator against free radical formation. In the present investigation, we prepared six different methanolic Fractions viz Fractions 1, Fractions 2, Fractions 3, Fractions 4, Fractions 5 and Fractions 6 from the methanolic extract of *Acacia catechu* stem bark. Then we check the *in vitro* antioxidant activity of different extracted fractions compared with standard ascorbic acid. Based on the high potent antioxidant capacity Fraction 3 were further evaluated the antiproliferative effect on MCF-7 breast cancer cell line. However, MTT assay revealed that, methanolic extracted Fractions 3 had potent cytotoxic effects on MCF-7 cells in the IC₅₀ value at 49.86 µg/mL. The study suggested that, the isolated Fraction 3 form the stem bark of *Acacia catechu* act as preventive agent for free radical formation and also cytotoxicity effect on MCF-7 cell line under laboratory condition.

Key words: Antioxidant activity, cytotoxicity MCF-7 cell line.

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INTRODUCTION

Cancer is one of the most important global issues that have an impact on people of all genders, ages, and races. Metastasis is a cellular phenomenon marked by abnormal cellular proliferation and multiple cell invasions into various body areas. In the United States today, breast cancer is one of the major causes of mortality. According to previous research, 85 percent of women with breast cancer had poor sleep quality, which has been linked to low self-esteem, pain, and despondency.^[1,2] Herbal medicine has developed in an unexpected way and it is now a viable therapeutic option for a variety of conditions with no side effects. Various plants such as *Casuarina equisetifolia*, *Aspergillus niger*,^[3]

Convolvulus arvensis^[4] and *Gymnema sylvestre*,^[5] have recently been discovered to exhibit anticancer activities. Women cancer development has been extensively studied.^[6] In the Kingdom of Saudi Arabia (KSA), breast cancer was the ninth leading cause of death among women in 2010 with the number of cases rising due to the population increase and ageing.^[7]

Breast cancer is a severe health problem when compared to cervical cancer,^[8] later, which has been identified as the top causes of mortality.^[9] Other cancer-related deaths may also rise in the next decades, WHO (2017). Although cancer management is used in chemotherapy, there are still significant side effects and indiscriminate toxicity issues.^[10] According to breast cancer for 30% of all novel cancer of cases in women each year.^[11] Chemotherapy in combination with radiation and surgical treatment is the conventional strategy for increasing medication concentrations while minimizing toxicity and side effects for patients.^[12]

Acacia catechu is a wild species of plant. It is a traditional medicinal herb that is most commonly found in Asia. The

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investigation of traditional medicines pharmacological activity has been made easier because of technological advancements. Among them *Acacia catechu* which in India is widely utilized to produce pharmaceutical effects, typically calls Katha or Karangali (Family: Leguminosae, Subfamily: Mimosiasae). Similarly they are also treated for passive diarrhoea with cinnamon or opium alone.^[13] This stem bark extract has several of pharmacological properties and including anti-inflammatory properties.^[13] The traditional treatment method for many diseases, which is based on the availability of therapeutic plants in a variety of parts of the World, provides a wealth of knowledge for drug development. Breast cancer is a difficult disease for oncologists to efficiently treat without causing any adverse effects. The traditional knowledge of medicinal plants provides medications that are more compatible cost-effective and have no harm.^[14]

There are no scientific reports available on this plant and also no anticancer studies have been so far conducted. Hence, an attempt has been made to observe the anticancer activity of *Acacia catechu* methanolic stem bark extract of against breast cancer cell line.

MATERIALS AND METHODS

Collection and Identification

Acacia catechu stem bark plant was collected in and around Kollihills, Namakkal district, Tamil Nadu, India (11 36'0"N, 78 33'0"E). The plant was recognised by the Taxonomist at Annamalai University, Department of Botany in Annamalai Nagar, India. Stem bark material was washed and air dried for fifteen days at room temperature. The stem bark was grind into a fine, coarse powder in a mixer grinder.

The plant material was collected and dried in the shady place for a week before being crushed and powdered. 100 gm of powder was weighed and soaked in methanol for 3 days (72 hr), after which it was placed into a soxhlet apparatus and allowed to boil for 1 hr. This dried methanolic extract of *Acacia catechu* was used for further studies.

Activity of Antioxidant

DPPH assay

The free radical scavenging activity was measured using the stable radical DPPH using the method of Kikuzaki H, *et al.*^[15] In this experiment 1.0 ml of 0.1 mM DPPH in methanol was combined with 1.0 ml of the *Acacia catechu* extract (varying from 2 to 10 mg/ml). The reaction mixture was vigorously agitated and kept at room temperature for 30 min in the dark. After then,

the absorbance was measured at 517 nm against a blank ascorbic acid standard. The reaction mixture's lower absorbance indicated better free radical scavenging activity. The *Acacia catechu* DPPH radical scavenging activity was calculated was using the following equation.

$$\text{Scavenging effect (\%)} = \left[\frac{(A_c - A_s)}{A_c} \right] \times 100$$

Thus, A_c is the absorbance of the control and A_s is the absorbance of the sample or standard.

Nitric oxide radical Scavenging activity

The process is based on the procedure in which sodium nitroprusside in plant extract solution at physiological pH spontaneously creates nitric which interacts with oxygen to make nitrate ions, which are diazotization with sulphanilic acid and then combined with N-naphtyl ethylene to from a red dye. The amount of nitric oxide produced by sodium nitroprusside was measured using the method of Marcocci L, *et al.*^[16] Phosphate buffer saline -100Mm, pH 7 one gram of buffer tablet is dissolving in 100ml of distilled water. Sodium nitroprusside- 10m 0.5gm of sodium nitroprusside was dissolved in 100ml of water with or without the plant extract light source (25W tungsten lamp). Sulphanilic acid -0.5% in 20%. The Glacial acetic acid 0.5g of sulphanilic acid was in 100ml of 20% glacial acid. Naphtyl Ethylene Diamine Dihydrochloride (NEDD) – 0.1% in 0.1 M Hcl. One gram of NEDD was dissolved in 0.1 M hydrochloric acid.

Percentage nitric radical scavenging activity calculated by the same formula that was used to calculate the DPPH radical-scavenging activity

Testing cytotoxicity of anticancer agents

The cytotoxic effect of *Acacia catechu* extract against MCF-7 cell line was calculated by a rapid colorimetric assay using the 3-(4, 5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide MTT compared to untreated control. The cells (1×10^5 per well) were insert in 96 well plates and incubated at 37°C with 5% CO₂. The cell attained confluence, different concentration of *Acacia catechu* extract was added and incubated for 24 hrs. *Acacia catechu* extract was repeated 3 times. After the procedure of incubation along with the sample 100 µl/well (5 mg/ml of 5% of MTT) was supplementary and visible below a microscope. 100 µl of solubilisation solution (DMSO) was added and mild blended in a rotary shaker for improving disintegration. The absorbance of each cell was estimated at 570nm using a microplate reader per user utilizing DMSO as clear. The IC₅₀ values were used to calculate the percentage of cell viability.^[17]

$$\text{Cell viability (\%)} = \left[\frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})}{\text{OD}_{\text{control}}} \right] \times 100$$

Where $\text{OD}_{\text{sample}}$ = absorbance of the treated sample, OD_{blank} = absorbance of medium + DMSO, and $\text{OD}_{\text{control}}$ = absorbance of non-treated sample.

The nonlinear regression graph between percent of cell inhibition and log concentration was plotted using GraphPad Prism software to calculate the IC_{50} .

Statistical evaluation

The experiment were frequent three times and the result were given as mean \pm SD. A direct relapse assessment was used to determine the IC_{50} for each *Acacia catechu* extract.

RESULTS AND DISCUSSION

DPPH Activity of the fractions

The present result (Figure 1) denotes that methanolic extract of *Acacia catechu* was exhibited the extensive potential DPPH radical scavenging activity by comparing standard ascorbic acid. The DPPH radical scavenging activity of methanolic third fraction exhibited extensive potential activity of 96.11 % at 50 $\mu\text{g}/\text{ml}$. However, the standard ascorbic acid demonstrated the 97.57 % inhibition in the same concentration. We observed that significant changes that all the concentration; the third fractions were significantly prior than standard ascorbic acid in all concentration. On the other hand, the third fraction was significantly higher than other fraction like Fraction 1, Fraction 2, Fraction 4, Fraction 5, and Fraction 6. Even then, the radical scavenging activity of methanolic extracts Fractions was significantly difference with ascorbic acid ($P < 0.05$). It tends to increase with increasing its concentration. The biological functions of phytoconstituents may be related to their antioxidant properties.^[18] Because it has an unconnected electron, DPPH is not affected by

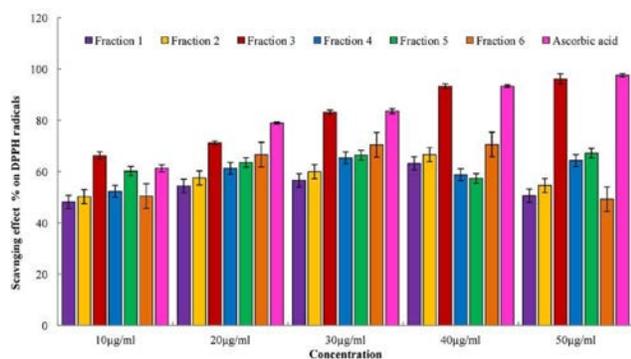


Figure 1: DPPH radical scavenging activity of *Acacia catechu*.

radical metals and enzyme inhibition, and it has a strong absorption maximum at visible spectroscopy at 517 nm.^[19,20] Such activity has been reported in many plant extracts, *Acacia catechu*,^[21] *Caralluma adscendens* var.,^[22] *Ocimum basilicum*^[23] and *Tabernaemontana divaricate*.^[24]

Nitric oxide assay

Nitric radical scavenging assay was carried out from the methanolic Fractions viz., Fraction 1, Fraction 2, Fraction 3, Fraction 4, Fraction 5, and Fraction 6 from a dried stem bark of *Acacia catechu* at 10 $\mu\text{g}/\text{ml}$ to 50 $\mu\text{g}/\text{ml}$ concentration. The (Figure 2) shows that methanolic extract of *Acacia catechu* Fraction exhibited the potential radical scavenging activity against nitric oxide. The maximum free radical scavenging capacity were interpolated from Figure 2 given that methanolic extract of Fraction 3 were potential radical scavenging activity of 83 % and then that of 93 % in standard ascorbic acid at 50 $\mu\text{g}/\text{ml}$ concentration. The radical scavenging of methanolic extract of Fractions was less than that of ascorbic acid. This will increase with increasing concentration. As like that of DPPH, the Nitric radical scavenging activity was also similar that, the methanolic extract of third Fraction was higher radical scavenging activity than that of other Fractions like Fraction 1, Fraction 2, Fraction 4, Fraction 5, and Fraction 6. Nitric oxide (NO) is produced by vascular endothelial cells, phagocytes, and certain brain cells from the amino acid L-arginine. Because of its unpaired electron, nitric oxide is classified as a free radical and exhibits significant reactivity with certain types of proteins and other free radicals. When nitric oxide reacts with superoxide radical, it forms a highly reactive peroxynitrite anion (ONOO⁻), which is toxic.^[25] Such activity has been reported in several plant extract *Ocimum gratissimum*,^[26] *Morus alba*,^[27] *Stevia rebaudiana*,^[28] *Uraria crinite*^[29] and *Arbutus unedo* L.^[30]

In contrast to the Fraction 3 was high potential radical scavenging activity in both DPPH and NO assay. On

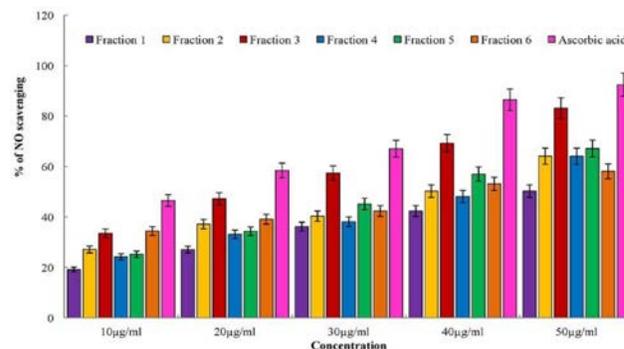


Figure 2: Nitric oxide scavenging activity of *Acacia catechu*.

the basis, we focused the methanolic extract form the stem bark of *Acacia catechu* Fraction 3 alone underwent further assessment for its *in-vitro* anti-oxidase potency in the MCF-7 (breast cancer cell line).

Cytotoxicity assay

The cytotoxicity of the methanolic extract form the stem bark of *Acacia catechu* Fraction 3 was assessed by using MCF-7 breast cancer cell line. A significant reduction of cells in activity was seen in a dose dependent manner. The expected result was observed in the potential cytotoxic effect on breast cancer cell line morphology with on IC_{50} value of $49.85\mu g/mL$ after 24 hr treatment (Figure 3). This was not seen in untreated MCF-7 control cell line. Under the phase contrast microscope view the morphological changes in both untreated and untreated MCF-7 cells were examined. Untreated MCF-7 (control) cells revealed a smooth, flattened morphology with high monolayer cells and a uniform cell membrane (Figure 4). At the same time, treated *Acacia catechu*

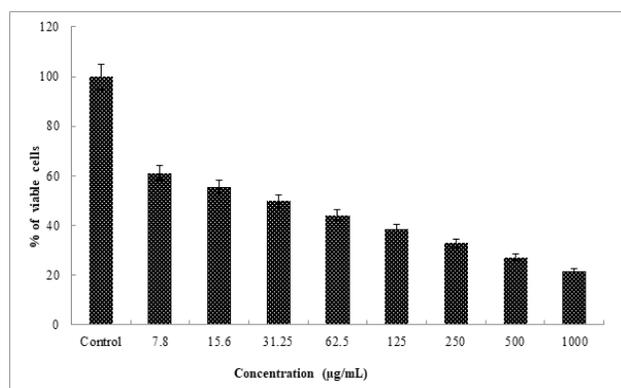


Figure 3: Effect of *Acacia catechu* on MCF-7 cells anticancer activity.

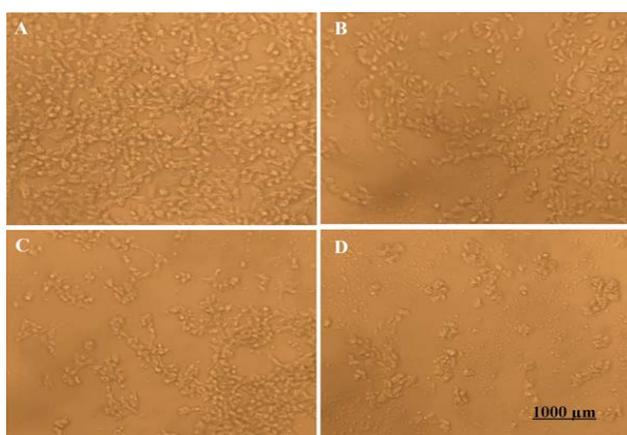


Figure 4: IC_{50} value crude extract of *Acacia catechu* against MCF7 breast cancer cell line. (A) Untreated MCF7 cell line; (B) treated extract of *Acacia catechu* at $7.8\mu g/ml$ (C) treated extract of *Acacia catechu* at $31.2\mu g/ml$; (D) treated extract of *Acacia catechu* at $1000\mu g/ml$.

Fraction 3 cells exhibited retraction, circled, detached from the cell surface and suspended cells were apparently accumulated. The increase in the number of apoptosis cells clearly explains the activation of programmed cell death.^[31,32] These results suggest that *Acacia catechu* may induce cell death in MCF-7 cells and that our findings are consistent with previous reports.^[33,21]

CONCLUSION

We concluded the present study showed that methanolic extract of *Acacia catechu* stem bark isolated from six different Fractions here investigated to demonstrated promising effect on antioxidant capacity by using DPPH and NO assay. In addition, the Fraction 3 acts as high potential antioxidant activity and it exhibited the excellent anti-cancer activity against the MCF-7 cell line. Thus it's obvious and comprehensive isolation of cancer leading molecules and further study were needed to investigate this phenomenon and to elucidated the mechanism of cytotoxicity in the MCF-7 cells of the Fraction 3, further study were underway.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ONOO: Peroxynitrite; **NEDD:** Naphthyl Ethylene Diamine Dihydrochloride; **HCL:** Hydrogen chloride; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl-hydrate; **CO₂:** Carbon dioxide; **DMSO:** Dimethyl sulfoxide; **NO:** Nitric oxide; **NFST:** National Fellowship for higher Education ST Students; **MOTA:** Ministry of Tribal Affairs.

SUMMARY

The MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was used to assess the antiproliferative and antioxidant properties of *Acacia catechu* against the *in vitro* breast cancer MCF-7 cell line. The *Acacia catechu* stem bark was extracted in stages using polar solvents like methanol.

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