



A Comparative Study between Plant and Callus Extracts of *Abutilon indicum* (L.) Sweet: Antioxidant, Antibacterial, Antidiabetic and Anti-Proliferative Activity

A. Ayisha Sireen¹ and J. Anbumalarmathi^{1*}

¹Department of Biotechnology, Stella Maris College (Autonomous), Chennai- 600086, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author AAS performed the statistical analysis and wrote the protocol. Author JA designed the study, managed the analyses of the study and the literature searches. Authors JA and AAS wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2020/v29i930220

Editor(s):

(1) Dr. Chunying Li, Georgia State University, USA.

Reviewers:

(1) Rejane Maria da Silva, Secretaria de Educação de Pernambuco, Brazil.

(2) Ondo Joseph Privat, Université des Sciences et Techniques de Masuku, Gabon.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59306>

Original Research Article

Received 01 June 2020
Accepted 05 August 2020
Published 04 November 2020

ABSTRACT

Abutilon indicum is considered to be used in the traditional system of medicine. It is found in tropical and subtropical regions of the world. It is used to treat various diseases. This plant does not cause any side effects to humans. As the plant has wide variety of medicinal properties, the present study aimed to compare between plant and callus extract of *Abutilon indicum* (L.) sweet for antioxidant, antibacterial, antidiabetic and anti-proliferative activity. The highest percentage of callus induction (89.50%) and callus weight (1.26 g) was observed in T₅ (MS + 2, 4-D (2.5 mg/l) + BAP (2 mg/l) and T₈ [IBA (4 mg/l)] respectively. Phytochemical analysis of aqueous and ethyl acetate extracts of *A. indicum* in vivo plant and in vitro grown callus showed the presence of alkaloids, flavonoids, phenols, carbohydrates, glycosides, protein, terpenoids, saponins, tannins and coumarin. The total phenolic content was high in aqueous extract of callus (30.68 mg TAE/g). Maximum DPPH radical scavenging activity was found in aqueous extract of callus (86%) with IC₅₀ value of 68.49 µg/ml. FT-IR analysis of aqueous extract of *A. indicum* plant and callus showed the

*Corresponding author: E-mail: amalarmathi@gmail.com;

presence of characteristic stretching at 2930.28 and 2927.75 indicating the presence of C-H stretching respectively. GC-MS analysis revealed the presence of 17 compounds in ethyl acetate plant extract, whereas 7 compounds in ethyl acetate callus extract such as tetradecane, 1-chloro, Sulfurous acid 2-propyltridecyl ester and 1-ethyl-3-[2-(octadecylthio) ethyl] thiourea. The ethyl acetate extracts of callus and plant and was found to be effective against *Bacillus subtilis* (3.1 mm) and *Staphylococcus aureus* (2.9 mm). Maximum α -amylase inhibitory activity was observed in aqueous callus extract (32.65%) with IC_{50} value of 833.61 μ g/ml. HeLa cell viability was found to be 26.8% and 21.8% in plant and callus extract respectively.

Keywords: *Abutilon indicum*; callus; phytochemicals; antioxidant; antidiabetic; cell viability.

1. INTRODUCTION

Abutilon indicum (L.) Sweet. (Family: Malvaceae) is a erect velvet-pubescent shrub with circular-ovate or heart shaped leaves with coarsely-serrate margins. This plant is found in hotter parts of India and also distributed in tropical and subtropical regions of the world. In Tamil Nadu it is commonly called as Thuthi. It has been extensively used in the traditional system of medicine. The plant is used as laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic and analgesic and used to treat leprosy, ulcer, headaches, gonorrhea, bladder infection, hemorrhoids, diabetes and menorrhoea. It also possess antidiabetic, anti-mycotic, antioxidant and antibacterial hepatoprotective, anti-diarrheal and anti-convulsant properties [1]. The plants are good source for the pharmaceuticals compounds for curing ailments of human being without any side effects. It possess phytochemical constituents such as asparagine, saponins, flavonoids, alkaloids, hexoses n-alkane mixtures and alkanol as main compound. The other constituents of the plant are β -sitosterd, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid, abutilona A, (R)-N-(1-methoxycarbonyl-2 phenylethyl)4-hydroxybenzamide, p-hydroxybenzoic, galacturonic, p- β -D-glycosyloxybenzoic and amino acid. It also contains essentials oil. Secondary metabolites like flavonoids and phenols are involved in antioxidant defense mechanism of plants [2].

Conservation of the species is very important as the *A. indicum* biomass is endangered due to environmental stress. Propagating the plant through tissue culture method is the solution for meeting the increased demand of the plant [3].

Hence, the present study was undertaken to develop *in vitro* propagation of *Abutilon indicum* using leaf explants, analyze phytochemical

constituents, study the antioxidant, antibacterial, antidiabetic and anti-proliferative activity of *in vivo* plant and *in vitro* grown callus of *A. indicum* (L.) Sweet.

2. MATERIALS AND METHODS

This study was conducted in Department of Biotechnology, Stella Maris College (Autonomous), Chennai during 2017-2019. The sample was collected from Semmozhi Poonga, Chennai and used for further investigation.

2.1 Sample Collection

The leaves of *Abutilon indicum* was collected from Semmozhi Poonga, Chennai.

2.2 Culture Media

Murashige Skoog culture medium (MS medium) [4] was supplemented with different growth hormones such as auxin 2,4-Dinitrophenylhydrazine (2,4-D), Indole-3-butyric acid (IBA) or its combination with cytokinin 6-Benzyl amino purine (BAP) for the callus initiation.

2.3 Preparation and Surface Sterilization of Explant

Leaf segments were excised from the healthy plant using sterilized surgical blade and brought to the laboratory. The leaf segments were washed thoroughly in tap water to remove the dust followed by distilled water. The leaves were cut into 1-1.5 cm [3]. Surface sterilization of explants (leaves) were done under aseptic condition in a laminar airflow. Explants were washed with autoclaved distilled water 3-4 times for 10 minutes followed by surfactant (tween-20: 2-3 drops/100 ml water) treatment for 5 minutes. It was again rinsed with autoclaved distilled water and followed by 70% ethanol for 30 seconds. Then, it was treated with mercuric chloride

(HgCl₂) 0.1% for 10 minutes followed by rinsing with 70% ethanol and thrice with autoclaved distilled water. After undergoing the process, the explants are ready for inoculation [5].

2.4 Inoculation of Explant

The explants (leaves) were inoculated horizontally on MS medium for culture initiation. The cultures were inoculated containing one explants per test tube and three explants per culture bottle. For each treatment, ten test tubes and six culture bottles were maintained [5]. The following different concentration of auxins and cytokinins (T₁-T₁₀) were incorporated in the MS-medium for callus initiation: T1: MS+ 1.5 mg/L 2, 4-D + 1 mg/L BAP, T2: MS+ 2.5 mg/L 2, 4-D + 1 mg/L BAP, T3: MS+ 4 mg/L 2, 4-D + 3 mg/L BAP, T4: MS+ 4 mg/L 2, 4-D + 4 mg/L BAP, T5: MS+ 2.5 mg/L 2, 4-D + 2 mg/L BAP, T6: MS+ 2 mg/L IBA, T7: MS+ 4 mg/L IBA, T8: MS+ 2 mg/L BAP + 4 mg/L IBA, T9: MS+ 1 mg/L BAP + 1.5 mg/L IBA and T10: MS+ 2.5 mg/L BAP + 2 mg/L IBA.

The tubes and culture bottles were shifted to the culture room at 25± 2°C under 16 hrs of photoperiod with light intensity of 2000 lux and 50-60% relative humidity [6].

2.5 Characteristics Observed

- Number of days for callus induction: The number days to callus induction was recorded from the day of inoculation to the first appearance of callus.
- Callus induction percentage: The callus induction percentage was recorded as the number of explants showing callus induction to the number of explants inoculated on the medium.
- Callus weight (g): Weight of callus was recorded after 5 weeks of inoculation and recorded as fresh weight [5].

2.6 Total Ash Content

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 2.0 g of powdered sample was weighed accurately and evenly distributed in the crucible. The content was shared over a heater and then burnt in a muffle furnace for 2 hours at 600±10°C. The crucible was allowed to cool in desiccators. The desiccated, cooled basin was then weighed with the ash [7].

$$\% \text{ Total ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

2.7 Preparation of Plant and Callus Extract

The whole plant was washed with tap water, followed by distilled water and allowed to shade dry. The dried plant materials were then ground into a coarse powder in a mechanical blender and stored in air tight container. Plant powder (25 g) was extracted in a Soxhlet with 250 ml of ethyl acetate. The collected extracts were evaporated using rotary evaporator. Aqueous extract was prepared by boiling plant material in water and was filtered. Callus was dried in shade and then made into powder and extracts were prepared using aqueous and ethyl acetate. Extracts were stored in air tight bottles at 4°C until use [8].

2.8 Phytochemical Analysis

Aqueous and ethyl acetate extracts of *in vivo* plant and *in vitro* grown callus of *A. indicum* were used to determine the presence of various phytochemicals [7].

2.9 Total Phenolic Content

The total phenolic content of aqueous and ethyl acetate extract of *A. indicum* plant and callus were estimated using Folin-Ciocalteu method. One (1) ml of the extracts (1 mg/ml) were added into empty test tube to which 5 ml of Folin-Ciocalteu reagent was added. To this 4 ml of 7.5% sodium carbonate was added. This was incubated for 30 minutes at 20°C. The absorption was measured at 765 nm. Total phenolic content was expressed as Tannic acid Equivalents in milligrams per gram dry material. For the preparation of standard Tannic acid solution, 5 mg of Tannic acid was dissolved in 1 ml of distilled water and stored as stock. Working standards was made in aliquots (0.01-0.05 mg/ml) using stock [7].

2.10 Antioxidant Activity

The DPPH radical scavenging activity was done in aqueous and ethyl acetate extract of *A. indicum* plant and callus. One (1) ml of extract (0.2-1 mg/ml in ethanol) was added to the 1ml of 0.01 mm of DPPH solution. One (1) ml of ethanol and 0.95 ml of Tris HCL was added to it and incubated at 37°C for 30 minutes in dark. Absorbance was measured at 517 nm. Ascorbic acid was used as reference standard [7].

Percentage (%) inhibition of DPPH activity was calculated using the formula,

$$\% \text{ inhibition} = \frac{(A_B - A_A)}{A_B} \times 100$$

Where, A_B - absorbance of the control
 A_A - absorbance of the sample

2.11 Fourier Transform-Infrared Spectroscopy (FT-IR) Analysis

Aqueous extract of plant and callus of *A. indicum* were subjected to FTIR analysis as it showed better antioxidant activity. Fourier Transformer Infrared (FT-IR) spectrophotometer (Bruker) analysis was carried out between 400- 4000 cm^{-1} to identify the functional groups present in the extracts [9].

2.12 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

Ethyl acetate extract of *A. indicum* plant and callus were subjected to GCMS analysis at VIT, Vellore. Identification of specific components was done by comparing mass spectra and the retention times with the data provided with the NIST library and confirmed. The peak area percentages of the chromatography represent the abundance of the compounds in the extract [9]. The peak area percentage was calculated by the formula,

$$\text{Peak area percentage (\% of component)} = \frac{\text{area under peak}}{\text{total area}} \times 100$$

2.13 Antibacterial Activity

Three bacterial strains *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were used to determine antibacterial activity. All the test organisms were maintained on nutrient agar slants in the laboratory. Antibacterial activities of the aqueous and ethyl acetate plant and callus extract of *A. indicum* determined by agar well diffusion method (Kirby Bauer et al. 1959). The culture suspension was adjusted by comparing with 0.5 Mc Farland turbidity standard. The plates were swabbed with 100 μl of the test organisms. A sterile cork borer was used to make wells of 5 mm diameters. DMSO 5% served as the negative control and different concentration of extract (50 $\mu\text{g}/\text{ml}$ and 100 $\mu\text{g}/\text{ml}$) were added into the respective wells. The plates were then incubated at 37°C overnight. The resulting zones of inhibition were measured

using a ruler calibrated in millimeters. The average of the triplicate reading was taken to be the zone of inhibition of the bacterial isolates [10].

2.14 Anti-Diabetic Activity

The α -amylase activity of aqueous and ethyl acetate extract of *A. indicum* plant and callus was determined.

2.15 Isolation of Wheat α - Amylase

Wheat flour (500 g) was added slowly with stirring to 2 liters of 0.2% calcium acetate solution at room temperature and stirred continuously for 2 hours. This suspension was then centrifuged at 4°C at 12000 rpm for 10 minutes. Clear brown supernatant was stored at 2°C to 3°C prior to heat treatment. Beta amylase was inactivated by heating the extract at 70°C for 15 minutes. Alpha amylase is resistant to this treatment at pH 6.5 and 8.0. The pH of the extract was adjusted to 6.6 with 4% Ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using water bath with continuous stirring. Extract was then cooled to 2°C to 3°C [11].

2.16 Acetone Precipitation

The crude enzyme extract was taken in a glass beaker and to it chilled acetone was added slowly, with continuous stirring upto 70% (v/v) concentration and kept at 20°C for 4 h to allow protein precipitation. The precipitates were then harvested by centrifugation at 10,000 rpm at 4°C for 30 minutes. The pellet obtained was resuspended at 34 mL of 20 mM phosphate buffer (pH 6) to allow the solubilization of proteins. The insolubilized proteins were then removed by centrifugation at 10,000 rpm at 4°C for 30 minutes. Supernatant when subjected to dialyzed for overnight against same buffer at 4°C. The protein content was estimated using Lowry method and enzyme activity were determined [12].

2.17 Confirmatory Test for α - Amylase

2.17.1 Starch hydrolysis test

Enzyme extract (1 ml) was added into 5 ml of 1% starch solution and heated for 10 minutes at 40°C. To this 1 ml of iodine solution was added. Colour change occurred through shades confirmed the presence of α - amylase [12].

2.17.2 Determination of wheat α -amylase inhibitor activity

Determinations of wheat α -amylase inhibitor activity for plant and callus extract were performed using the chromogenic DNS method. One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per minute under the assay condition (Ankita et al. 2015). Extracts (5 mg) were dissolved in 10 ml of DMSO reagent. The solution was made into aliquots (0.1, 0.2, 0.3 upto 0.5 μ g/ml). The volume was made upto 1 ml with sodium phosphate buffer. α -amylase enzyme (0.5 ml) was added to each tube. The tubes were incubated at 37°C for 10 minutes. After incubation, 0.5 ml of 1% starch was added. Again incubated for 15 minutes at 37°C. After incubation, 1 ml of 3-5 Dinitro Salicylic Acid (DNS) was added to all tubes. All tubes were placed in boiling water bath for 10 minutes water was used as blank. Absorbance was read at 540 nm [6].

2.18 Antiproliferative Analysis [9]

Cell viability was determined in aqueous extract of *A. indicum* plant and callus.

Sub culture of cell line: Concentration of the cell suspension was adjusted by adding 100 μ l of a 5×10^5 cells (HeLa cells)/ml solution to each well and incubated for 24 - 48 hours in CO₂ incubator.

Drug treatment: 10 μ l of drug dissolved in DMSO was added to each well. It was placed on a shaking table at 150 rpm for 5 minutes, to thoroughly mix the samples into the media. It was then incubated at 37°C, 5% CO₂ for 12 - 24 hours.

MTT assay: 2 ml of MTT solution was added per 96 well plate at 5 mg/ml in DMSO/PBS. 20 μ l of MTT solution was added to each well. It was placed on a shaking table at 150 rpm for 5 minutes and incubated at 37°C, 5% CO₂ for 4 hours for MTT metabolization. Formazan (MTT metabolic product) was resuspend in 200 μ l of DMSO and placed on a shaking table at 150 rpm for 5 minutes. Optical density was read at 560 nm and subtract background at 670 nm, optical density should be directly correlated with cell quality. The cell density and percentage cell viability was calculated using the following formula.

$$\text{Cell density} = \text{OD Sample} - \text{OD blank}$$

The average cell density of triplets:

$$\text{Percentage of Cell viability (\%)} = \frac{\text{OD sample}}{\text{OD Control}} \times 100$$

3. RESULTS AND DISCUSSION

The present study was carried out in *A. indicum* plant and callus extracts. This study would help us to find the presence of various phytochemical compounds in plant and callus extract, which will help us in the future, development of medicines in pharmaceutical industries.

3.1 Callus Induction

The callus initiation was observed after 3 to 6 days of inoculation in 2, 4-D + BAP and after 7 to 10 days of inoculation in IBA. The complete proliferation was observed after 4 weeks of incubation. The highest percentage of callus induction (89.50%) was observed in T₅ (MS + 2, 4-D (2.5 mg/l) + BAP (2 mg/l) and callus weight of 1.26 g in T₈ (IBA (4 mg/l) (Fig. 1). Similarly, [13] reported callus induction from leaf explant in MS medium supplemented with 4.52 μ M 2,4-D and 8.88 μ M BAP. This method ensures the successful proliferation of callus from *A. indicum* which as a wider application in the field of medicine.

3.2 Total Ash Content

Total ash content of *A. indicum* plant powder were found to be 10.5%. Similar result was obtained by Sharma and Naveen [14] in *A. indicum* root was found to be 13.26%. Measuring ash content is important because mineral matter may be the cause of pharmacological effect.

3.3 Phytochemical Analysis

Aqueous extract of *A. indicum* plant and callus showed the presence of alkaloids, flavonoids, phenols, carbohydrates, glycosides, protein, terpenoids, saponins, tannins and coumarin. Ethyl acetate extract of *A. indicum* plant and callus showed the presence of alkaloids, flavonoids, phenols, carbohydrates, glycosides, terpenoids, saponins, and coumarin. Out of the two solvents used aqueous extract was observed to be best solvent for extraction of plant and callus. This study helps to determine the bioactive profile of plant and its therapeutic importance. Similar study was done by [15] in petroleum ether, benzene, ethyl acetate, methanol and ethanol in root and stem showed the presence of alkaloids, coumarin, flavonoids,

phenol, quinine, saponin, tannin, terpenoids, carbohydrates, protein, glycosides, xanthoprotein and fixed oil.

3.4 Total Phenolic Content

Phenols are important bioactive compound for antioxidant activity, were found to be present in all the extracts of *A. indicum* in minimal concentrations. The highest phenolic content

was observed in aqueous callus extract of *A. indicum* (30.68 mg TAE/g) (Fig. 2), followed by ethyl acetate extract of *A. indicum* callus (26.83 mg TAE/g) (Fig. 6). On comparing the total phenolic content of aqueous and ethyl acetate extract of plant and callus, the callus extract of aqueous showed highest phenolic content. Similar study was done by [16] in ethanol extract of *A. indicum* which showed highest phenolic content (64.6 mg/gm of gallic acid).

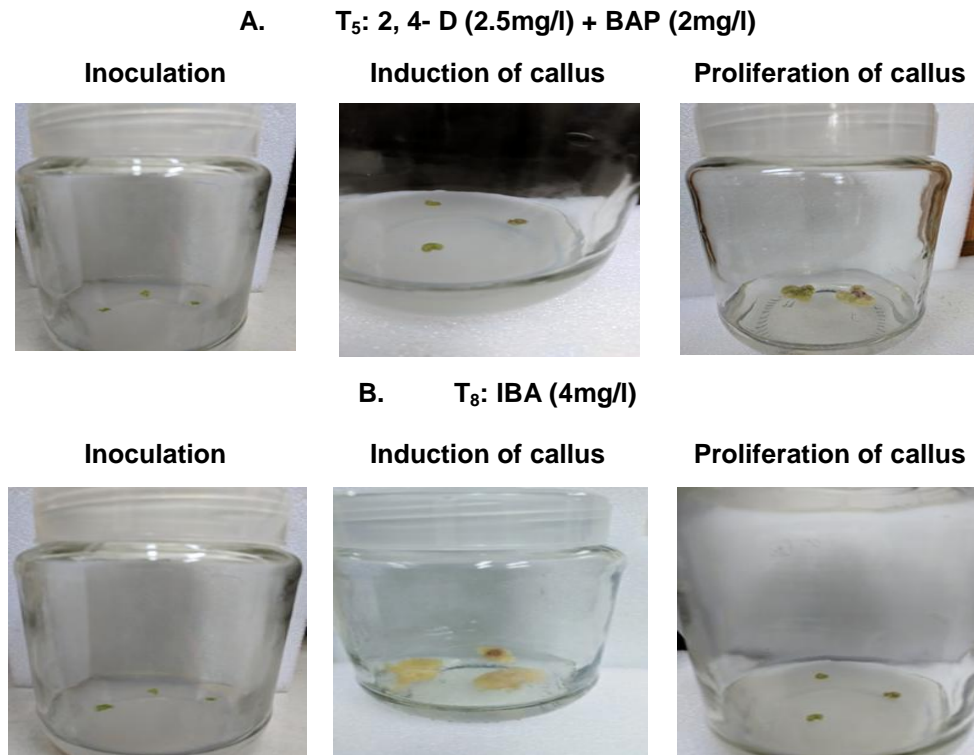


Fig. 1. Callus induction from leaf explant of *A. indicum* on MS media supplemented with T₅: 2, 4- D (2.5mg/l) + BAP (2mg/l) and T₈: IBA (4mg/l)

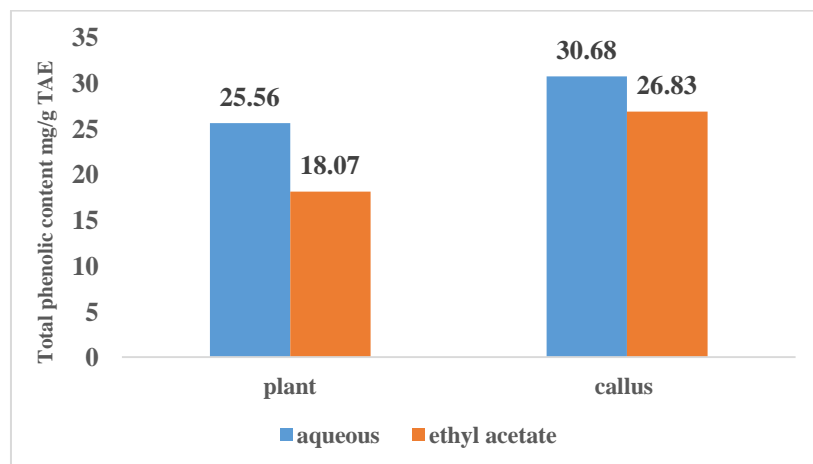


Fig. 2. Total phenolic content of aqueous and ethyl acetate plant and callus extract of *A. indicum*

3.5 Antioxidant Activity

DPPH radical scavenging activity was determined for aqueous and ethyl acetate extract of *A. indicum* plant and callus. On comparing DPPH scavenging activity of aqueous and ethyl acetate plant and callus extract, the highest DPPH radical scavenging activity was observed in aqueous callus extract at 86% with IC_{50} value of 68.49 $\mu\text{g/ml}$. The lowest DPPH radical scavenging activity was observed in ethyl acetate extract of plant at 50% with IC_{50} value of 117.69 $\mu\text{g/ml}$ (Figs. 3 and 4). Similar study was done by [15] in root and stem ethyl acetate extract of *A. indicum* was found to be 30.92 $\mu\text{g/ml}$ and 31.46 $\mu\text{g/ml}$ respectively.

3.6 Fourier Transform-Infrared Spectroscopy (FT-IR) Analysis

The aqueous plant extract of *A. indicum* found to possess peaks ranging from 618.62 cm^{-1} to

3407.78 cm^{-1} has confirmed the presence of functional groups such as primary and secondary amines and amides, alkane, carboxylic acid, alkyne, conjugated alkene, nitro compound, fluoro compound, sulformaide, aromatic ester, alkyl aryl ether, secondary and primary alcohols, amine, alkene and halo compound (Fig. 5).

The aqueous callus extract of *A. indicum* found to possess peaks ranging from 618.59 cm^{-1} to 3395.59 cm^{-1} has confirmed the presence of functional groups such as primary and secondary amines and amides, alkane, carboxylic acid, isothiocyanate, conjugated alkene, nitro compound, phenol, alkyl aryl ether, secondary and primary alcohols, alkene and halo compound (Fig. 6). This the first report in FTIR analysis of aqueous extract of *A. indicum* plant and callus. Similar study was reported by [17] in ethyl acetate extract of *A. indicum* showed the presence of various groups like alcohol, phenol, alkanes, amides and isopropyl groups.

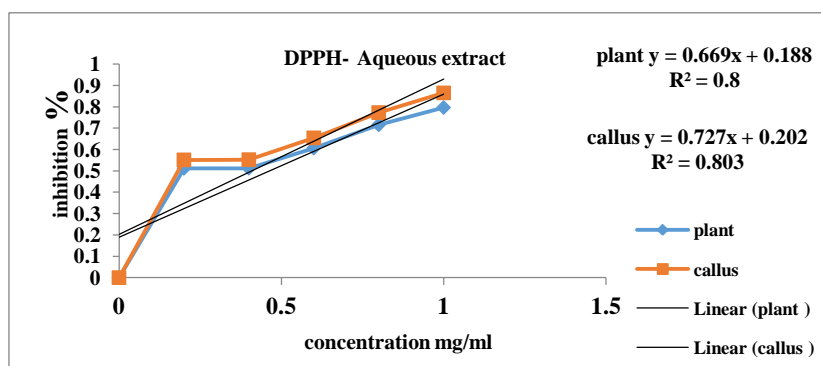


Fig. 3. DPPH radical scavenging activity of aqueous *in vivo* plant and *in vitro* callus extract of *A. indicum*

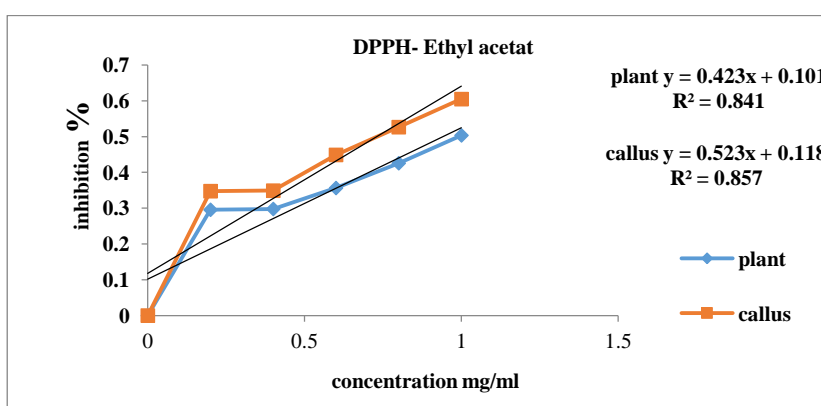


Fig. 4. DPPH radical scavenging activity of ethyl acetate *in vivo* plant and *in vitro* callus extract of *A. indicum*

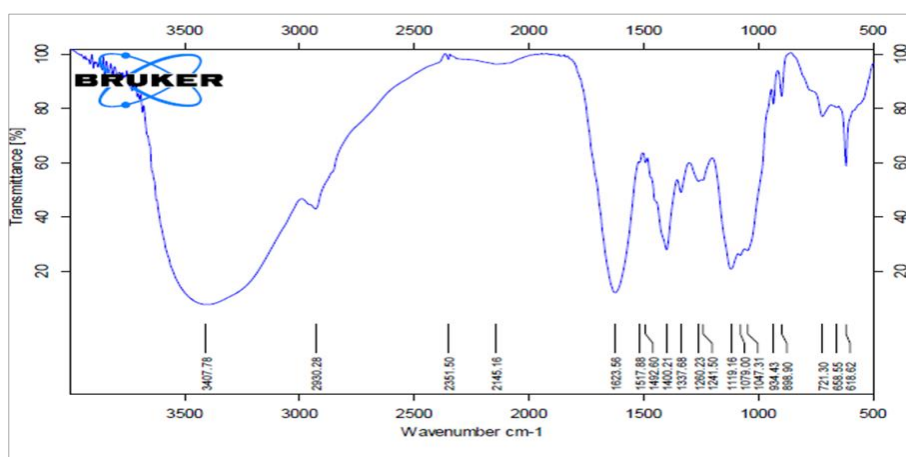


Fig. 5. FTIR chromatogram of aqueous *in vivo* plant extract of *A. indicum*

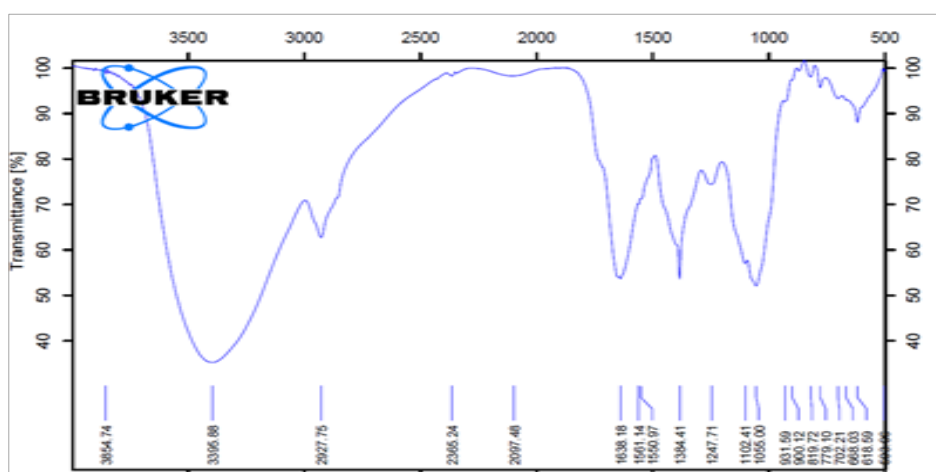


Fig. 6. FTIR chromatogram of aqueous *in vitro* callus extract of *A. indicum*

3.7 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

The ethyl acetate extract of plant found to possess 17 bioactive compounds, where the maximum peak area (13.577%) was covered by 1-Ethyl-3-[2-(octadecylthio) ethyl] thiourea followed by 1- Hexacosanol with the peak area of 12.316% (Fig. 8). The callus extract was found to possess 7 bioactive compound with the maximum peak area (37.795%) covered by L-AlanineN-Propoxycarbonyl-Heptadecyl Ester followed by 1-Ethyl,-3-[2-(octadecylthio)ethyl] thiourea with peak area 16.346% (Figs. 7 and 8). This study helps in the development of drugs for several disease like cancer, diabetes, etc. Similar study was done by [17] has reported the presence of 16 bioactive compounds in which the maximum peak area was covered by 3 Hydroxy

beta ionol in ethyl acetate extract of *A. indicum* leaves.

3.8 Antibacterial Activity

Among the aqueous and ethyl acetate extract of *A. indicum*, the ethyl acetate extract of *A. indicum* plant and callus was found to be effective antibacterial agent. The ethyl acetate extract of callus was found to be effective against *Bacillus subtilis* (3.1 mm) with 100 µg/ml, whereas ethyl acetate plant extract was effective against *Staphylococcus aureus* (2.9 mm) with 100 µg/ml (Table 1). Similar study was done by [18] in *A. indicum* leaf extract against *E. coli* and *Staphylococcus aureus*. Leaf extract was found to be effective against *Staphylococcus aureus* (2 mm) in very low concentration (2.5 µg/ml).

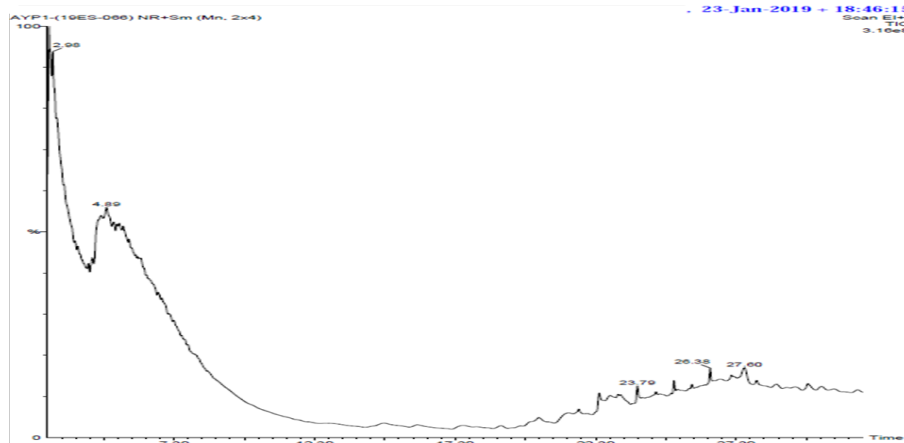


Fig. 7. GC-MS analysis of ethyl acetate *in vivo* plant extract of *A. indicum*

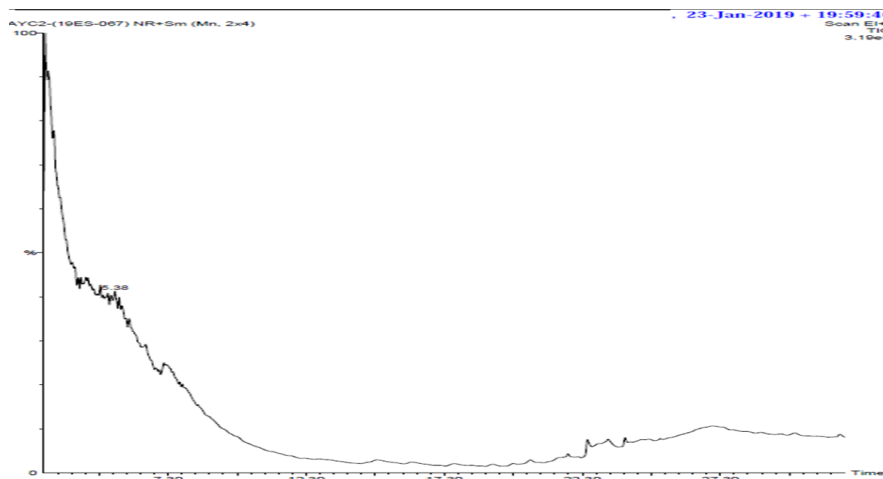


Fig. 8. GC-MS analysis of ethyl acetate callus extract of *A. indicum*

Table 1. Antibacterial activity of ethyl acetate extract of *in vivo* plant and *in vitro* callus of *A. indicum*

| Bacteria | Zone of inhibition (mm) | | | | Negative control |
|------------------------------|-------------------------|-----------|------------------------|-----------|------------------|
| | <i>In vivo</i> plant | | <i>In vitro</i> callus | | |
| | 50 µg/ml | 100 µg/ml | 50 µg/ml | 100 µg/ml | |
| <i>Bacillus subtilis</i> | 2.7 | 2.6 | 3 | 3.1 | - |
| <i>Escherichia coli</i> | - | - | - | - | - |
| <i>Staphylococcus aureus</i> | 2.3 | 2.9 | 2.4 | 2.3 | - |

3.9 Anti-diabetic Activity

This method of isolating α -amylase from wheat was found to be an efficient protocol with the protein content to be 22.2 mg/ml by calibrating against the standard curve of Bovine Serum Albumin. The presence of amylase enzyme was confirmed by the starch hydrolysis test showed an array of colour change on addition of amylase enzyme to the starch-iodine complex. Aqueous

callus extract has shown 32.65% inhibition at the concentration of 500 µg/ml with the IC₅₀ value of 833.61 µg/ml, followed by ethyl acetate extract of callus to be 29.35% with IC₅₀ value of 937.74 µg/ml. The aqueous extract of callus was found possess the highest α -amylase inhibitory activity (Figs. 9 and 10). Hence aqueous extract of callus can serve as a potent antidiabetic agent in future therapeutic areas. This is first report in α -amylase inhibitory activity of *A. indicum* in

aqueous and ethyl acetate extract of plant and callus. Similar study was done by [19] in ethanol extract of *A. indicum* root which was found to be a potent inhibitor of α - amylase (191.64 $\mu\text{g/ml}$) and α - glycosidase (207.13 $\mu\text{g/ml}$) which help to reduce postprandial glucose level.

3.10 Antiproliferative Analysis

Aqueous extracts of *in vivo* plant and callus of *A. indicum* was studied for inhibitory property on

HeLa cell lines by MTT assay. This study revealed that *in vivo* plant has inhibitory percentage of 26.8% whereas callus has 21.8% as inhibitory percentage against HeLa cells (Fig. 11). Thus it shows *in vivo* plant and *in vitro* callus has more potential to inhibit HeLa cells. Similar study was reported by [20] in methanolic extract of *Abutilon indicum* using SK-MEL-28 and NCI-H23 cell line. Inhibitory percentage of *A. indicum* in SK-MEL-28 is 57.52% and NCI-H23 is 17.29% at 5 mg/ml concentration.

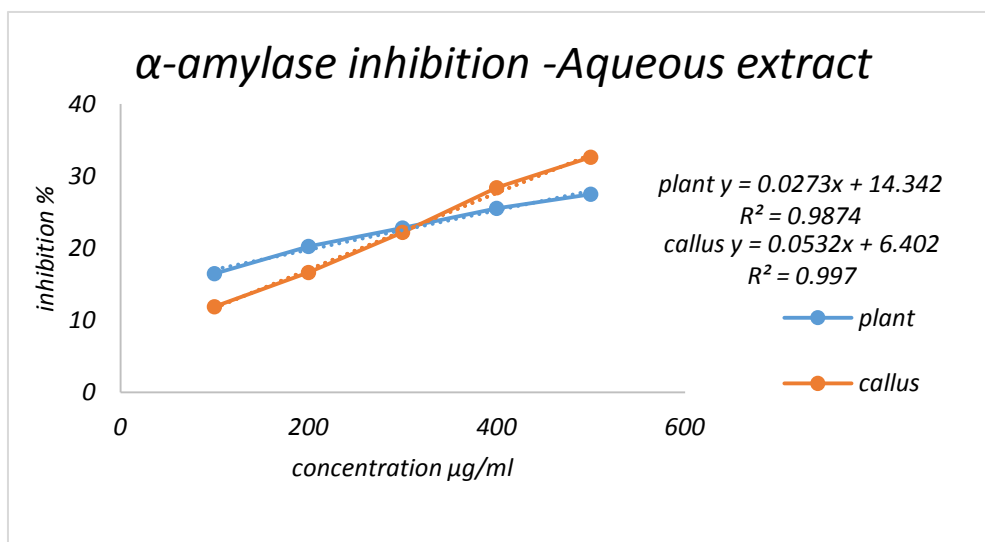


Fig. 9. α - amylase inhibitory activity of *in vivo* plant and *in vitro* callus aqueous extract of *A. indicum*

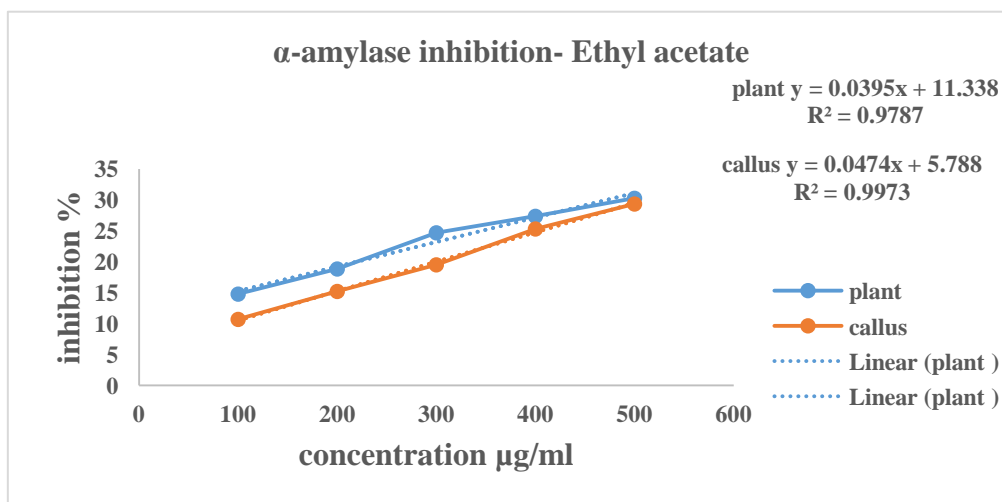


Fig. 10. α - amylase inhibitory activity of ethyl acetate *in vivo* plant and *in vitro* callus extract of *A. indicum*

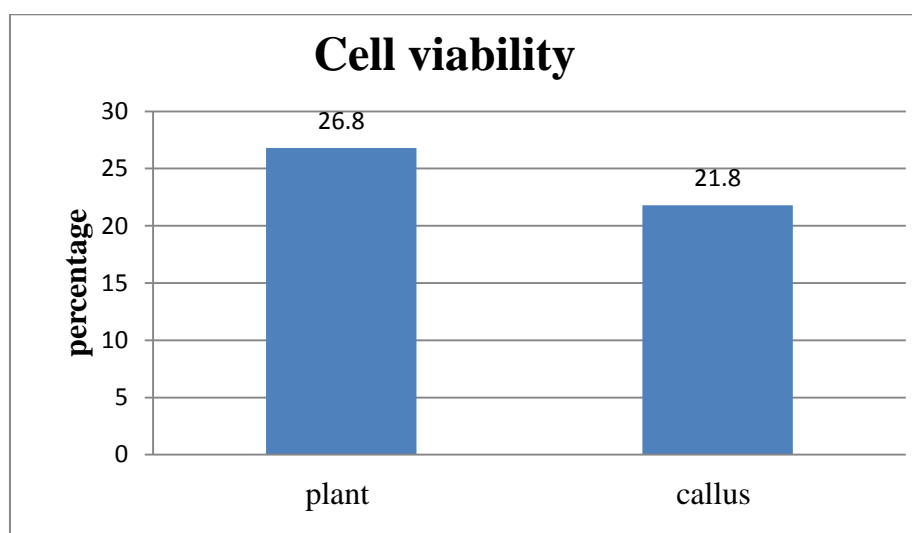


Fig. 11. Cell viability of *A. indicum* aqueous extract of plant and callus

4. CONCLUSION

Callus was initiated from leaf explants of *A. indicum* (L.) in MS media supplemented with 2, 4- D (2.5 mg) + BAP (1 mg) and IBA (4 mg/l) with 89% efficiency. The phytochemical screening of aqueous and ethyl acetate extract of both *in vivo* plant and *in vitro* callus revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, glycosides, protein, terpenoids, saponins, tannins and coumarin. Both *in vivo* plant and *in vitro* grown callus of aqueous and ethyl acetate extracts possess potent antioxidant activity. FT-IR and GCMS analysis revealed the presence of major compounds in both plant and callus extracts. The ethyl acetate extracts of callus and plant and was found to be effective against *Bacillus subtilis* and *Staphylococcus aureus*. Maximum α -amylase inhibitory activity was observed in aqueous callus extract. Antiproliferative analysis showed a considerable amount of inhibitory percentage against HeLa cells. Thus, the present study revealed the presence of various bioactive compounds indicating that this plant and callus extract compounds can be used as therapeutic drugs.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Principal and the Management of Stella Maris College for the research facilities provided. The authors acknowledge DST – FIST for FT-IR facility in CRIST lab, Stella Maris College (Autonomous), Chennai.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Joyti RR, Manorama M, Ritarani D, Santi Lata Sahoo. *In vitro* micropropagation of *Abutilon indicum* L. through leaf explant. *Plant Tissue Culture and Biotech.* 2009;19(2):177-184.
- Rajalaxmi N, Ashok P, Luna Samanta, Santilata Sahoo. Phytochemical analysis of antioxidants from *Abutilon indicum* (L.) and *Paederia foetida* (L.) supported by TLC, FTIR and NMR studies. *International Journal of Science and Research.* 2013;4(5).
- Sudarshana MS, Nissar AR, Grish HV. *In vitro* regeneration potentials of the medicinal plant *Abutilon indicum* (L.) Sweet. *African Journal of Biotechnology.* 2016;15(12):472-480.
- Toshio Murashige, Folke Skoog. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum.* 1962;15(3):473-497.
- Sujithra Devi R, Anbumalarmathi J, Aruna Sharmil S, Santhiya KR. Callus induction and micropropagation in *Phyllanthus amarus*. *International Journal of Herbal Technology.* 2016;1(1):23-26.
- Ankita R, Kalpesh B. *In vitro* shoot multiplication from nodal explants of *Coccinia grandis* (L.) Voigt and its

- antidiabetic and antioxidant activity. Asian Journal of Biological Sciences. 2015;8:57-71.
7. Divya A, Anbumalaramathi J, Aruna Sharmili S. Phytochemical analysis, antimicrobial and antioxidant activity of *Clitoria ternatea* blue and white floral leaves. Advances in Research. 2018;14(5):1-13.
 8. Syed ZS, Krishna B, Kandukurivasu, Singara Charya MA. Antimicrobial activity of the fruit extracts of *Coccinia indica*. African Journal of Biotechnology. 2009;5(1):1-7.
 9. Mercy Madhumitha K, Anbumalaramathi J, Aruna Sharmili S, Nandhini G, Shanmuga Priya. A comparative study of *In vivo* plant and *In vitro* callus extracts of *Centratherum punctatum* Cass. Annual Research & Review in Biology. 2020;35(3):1-13.
 10. Nandhini G, Anbumalaramathi J, Mercy Madhumitha K, Shanmuga Priya G. A comparative study between plant and callus extracts: Analysis of phytochemical, antioxidant, antibacterial and anticancer properties of *Ormosia sennoides* (Wild.) DC. International Journal of Scientific Research in Biological Sciences. 2020;7(1):95-103.
 11. Jayalakshmi V, Anbumalaramathi J, Arunasharmili S. *In vitro* callus induction of *Coccinia indica* (W. and A) and analysis of its phytochemical, antimicrobial, antioxidant and α -Amylase inhibitory activity- A comparative study. International Journal of Biochemistry Research & Review. 2019;28(3):1-16.
 12. Chavan, Wadkar CM. Isolation, purification and characterization of alpha amylase from *Phaseolus aconitifolius*. Journal of Advanced Drug Delivery. 2014;1(3):114-121.
 13. Sen S, Jogeswar P. *In vitro* organogenesis of *Abutilon indicum* (L.) sweet from leaf derived callus and assessment of genetic fidelity using ISSR markers. The Journal of Horticulture Science and Biotechnology. 2018;94(1):70-79.
 14. Surendra Kr Sharma, Naveen G. Preliminary phytochemical and pharmacognostic profile of *Abutilon indicum* Linn. root. Derpharmacologia Letter. 2010;2(5):308-315.
 15. Evanjaline R, Mohan VR. Screening of phytochemicals, *in vitro* antioxidant and antibacterial activities of stem and root extracts of *Abutilon indicum* (L.) SW. International Journal of Pharmaceutical Sciences Review and Research. 2017;43(1):75-83.
 16. Srividya AR, Dhanabal SP, Jeevitha S, Vishnu Varthan VJ, Rajesh R. Relationship between antioxidant properties and chemical composition of *Abutilon indicum* Linn. Indian Journal of Pharmaceutical Science. 2012;74(2):163-167.
 17. Saranya D, Sekar J. GCMS and FTIR analyses of ethyl acetate leaf extract of *Abutilon indicum* (L.) Sweet. International Journal of Advanced Research in Biological Sciences. 2016;3(2):193-197.
 18. Sujatha, Rajesh GG, Chandra SK, Nagma K. Antibacterial activity of *Abutilon indicum*. World Journal of Pharmacy and Pharmaceutical Science. 2015;4(9):946-949.
 19. Florenico Jr. V. Acre, Jeah E. Dela Concepcion, Katrina Mari C. Mayol, Gerard Lee L. See. *In vitro* α -amylase and α -glycosidase inhibition activity of *Abutilon indicum* (Linn 1836) root extracts. International Journal of Toxicological and Pharmacological Research. 2016;8(5):391-396.
 20. Srikanth, Karthik, Sirisha, SasikanthChitti. Evaluation of antioxidant and anticancer properties of methanolic extracts of *Abutilon indicum* and *Blumea mollis*. Journal of Pharmacy Research. 2012;5(4): 2373-2376.

© 2020 Sireen and Anbumalaramathi; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/59306>