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Research Article

ANALYSIS OF CHEMICAL COMPOUNDS IN INVITRO PROPAGATED PLANTS (L.)

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A B S T R A C T

Analysis of chemical compounds from invitro propogated ethanomedicinal plants *Bahunia purpurea, Cassia tora, Syzygium cumini, have used in* pharmaceutical Company and innovative new drug, which cure infectious & non infectious disease. Different tests were done for phytochemical analysis of the methanol and aqueous extracts. Invitro propagated plants i.e. *Bahunia purpurea, Cassia tora, Syzygium cumini through* the different concentrations of growth regulator, supplemented medium, photoperiod & temperature. In invitro propoogated plants, chemical constitutes (i.e. Saponin, Tannin, Carbohydrate Glycosides, Flavonids, Steroids, Terpenoids, Reducing Sugar, Indole Alkaloids) may be or may not be present The explants as seeds & nodes used for invitro regenerated plants done from seed germination on H.Strenght medium without sucrose and growth regulator, in the culture media require different photoperiod 12-12,10-14,8-16, Most suitable 8-16 /light – dark for seed germination. However in invitro multiplication of Plants *Bahunia purpurea, Cassia tora, Syzygium cumini* on full M.S. Medium supplemented with different growth regulator (1mg/ml). Auxin (0.5µl/ml) & cytokinin (2µl/ml) shows very good response for a shoot multiplication by the explants of *Bahunia purpurea*, *Syzygium cumini* as well as multiplication shoot of *cassia tora* growth require higher concentration cytokinin ((4µl/ml) and auxin was same. Several experiments have found that growth regulators (Auxin & Cytokinin) are important for shoot multiplication of *Bahunia purpurea, Cassia tora, Syzygium cumini* plants, We got another result where highest percentage of seed germination found in *Bahunia Purpurea,* then *Syzygium cumini,* and lowest percentage in *Cassia tora.* This research aims to create a reliable protocol for the Invitro propogated plants and analyse chemical compound by propgated plants.

INTRODUCTION

In research field a big hazard is to treat infectious diseases through alternative drugs of ethanomedicinal plants. During the last two decades the appearance of undesirable side effects of certain antibiotics, has lead to the search of a new antimicrobial, antioxidant chemical compound through phytochemical analysis Followed with the goal of present work, analysis of phytochemical compound in Invitro propagated ethanomedicinal plants *i.e. Bahunia purpurea, Cassia tora & Syzygium cumini,(L.) (*D. Sripriya 2014) Various plants restore important chemical compound as primary metabolism & secondary metabolism (C. Kaniz W.S.2016) Primary metabolism plays an essential role in respiration, photosynthesis, growth development. Primary metabolisms are phytosterols, acyl lipids, nucleotides, amino acid & organic acid.

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Crozier A., Kamiya Y., Bishop G., *et al* (2000) Biosynthesis of hormone & elicitor molecules. In B.B. Buchannan, W. Gruissem & R.L. Jones (eds) Although secondary metabolites have key role in protecting plants from herbivores & microbial infection as attractant for pollinators, UV protectants, Signal molecules in the formation of nitrogen – fixing root nodules in legumes as well as use as dyes, fibers, oils, waxes, flavoring agents drugs & perfumes. They are viewed as potential source of new natural drugs, antibiotics, insecticides, & herbicides (Dewick 2002) unlike the traditional vitamins they are not essential for short-term well being, but there is increasing evidence that modest long-term intakes can have favorable impact on the incidence of cancers & many chronic diseases including cardiovascular disease Type 2 diabetes. The *Cassia tora* L. Syn is belong to family leguminacea & Sub family Caesalpiniaceae, it is an ayurvedic plant commonly wild crop in India & with have ethanomedicinal importance like paste of leaves & seeds are useful in healing wounds, itching ringworms, skin disease, leprosy, bronchitis, constipation, Cough Cardiac Disorders. Chan M.J. & peria L.M. (2001)

The genus of Syzygium cumini (L) forest tree is widely distributed in India & other tropical region of the world, commonly known as jamun is widely used in Ayurveda & other Indian folk medicines for the treatment of diabetes mellitus. A fairly fast growing species, it cans heights of up to 30 mtrs. & can live more than 100 years (Ashwini Kumar M.K.2015). Syzygium cumini (L) is an evergreen tropical tree belonging to the Myrataceae family & native to India, Nepal Bangladesh (Berkar S.P.A. 2009) The leaves & seed extracts which is traditionally (Berkar S.P.A. 2009) used in diabetes has a hypoglycemic action antibacterial & antidiarrea effect.(Ashwini Kumar M.K.2015) Strengthen the teeth & gums to treat leucorrhea, stomachalgia, fever , gastropathy stangury, dermopathy (Chaudhari AKN Pal S, G.A. 1990,D.E.,W.N. 1999, Elizabeth Margaret, A.S. 2015, G.L.P. 2006, Gawri S.S.,V.K. 2010). Plants are the potential source of natural antioxidants, antibacterial or phytochemical antioxidants are the secondary metabolites of plants. Antioxidant & antibacterial agents like tannins,flavonoids, phenols, polyphenols & nitric acid, scavengers of free radicals such as peroxidase hydrogen or lipid peroxyl thus inhibits the oxidative mechanism that lead to degenerate disease.(Walton & Brown 1999). *Bahunia purpurea* linn.(leguminosae) is a medium sized deciduous tree, sparingly grown in India. The plant is used traditionally in dropsy, pain rheumatism, convulsions, septicaemia. (sAsolker LV., Kakker KK, Chakre OJ, 2000).

Stem bark of *bahunia purpurea* in Malays known as pokok tapak was used by Indian, Srilankan, Pakistani community to treat Ulcer, wound glandular, swelling stomach, tumor, antitode to poison an astringent to treat diarrhea, antihelminths, leprocy, menstrual disorders of the rectum. According to reports *Bahunia purpurea* Contains Steroidal Glycosides, Terpenoids, lactones Flavonoids. Iribarren AM, Pomilo AB, 1983. *Bahunia purpurea* reported to exhibit various pharmacological activities, Such as anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, anti-proliferative, anti-inflammatory activity. (Shaliselvin CD, 2011., Zakaria ZA 2009., Harborn JB., 1984., Kokate CK.,1995)

According to Averre & gooding (2004), (Jones 1983; Bajaj 1986.)Tissue culture is one of the most important techniques being used now day's propogation of woody plants for the tree improvement & reforestation has been discussed as burning issue by several workers, rapid & pathogen free plants? Invitro plant regeneration is to depend many factors on which most essential are composition of the basic medium, growth regulators, gelling agent, light intensity, photoperiod, temperature, cultivation vessel & vessel covers (reed 1998). In plant tissue culture a plant part (explant) is cultured on a nutrient medium under sterile condition with the purpose of obtaining growth. (Beachy 1997) A explants requires a nutrient medium for growth, a nutrient medium is a watery solution of all the substance in the correct quantities relative to one another in such a way that the explants can grow (Murashige & Skoog 1962) depending upon species to be cultured the type of tissue culture medium is selected for plant regeneration from tissue & callus (M.S. Medium 1962) is the most commonly used basic tissue culture medium another media also available which include (linsmaier & Skoog 1965) half salt M.S. medium full salt strength B5 Gamborg medium which was originally devised for callus cultures. (gamborg *et,al*.) ishag et.al., (2009) excised the regenerated shoots & were rooted on Full &half strength M.S. medium without or with different level NAA, IAA, or IBA. Media contains 95% water, Macro element, microelement growth Hormones vitamins , amino acid, sugar & gelling agent etc & balance media with 0.1 % Hcl & Nacl. Plant growth regulator play fundamental role in growth & development (little & savidage 1987) as well as enhance cell division & expansion (Tahimoto & haradara 1984) concentration of cytokinin & Auxin Is required for multiple shoot proliferation in M.S. Media But inclusion of low concentration of auxin along with cytokinin (0.1 to 10mg/L) trigger the rate of shoot proliferation (Tsay *et.al* 1989). Different type of shoot developed from different explants (cotyledonary, node, shoot tip) excised from Invitro grown seedling gare the best response (agrawal & sardar 2003, siddique & anis 2007). The gibberllins have primordial function as their exogenous application counter balance the inhibition imposed by the absisic acid & also case an endogenous increase of gibberllic acid which plays a key role in seed germination. (killer 1962, Khan 1971, Metiver 1979). Agar produced from seaweed is the most common type of gelling agent & is ideal for routine application (Murashige & skoog 1962). A success full tissue culture protocol starts with effective explants sterilization (dodds & Roberts 1985). Maina *et.al* (2010) evaluated commercial bleach Sodium hypo chloride (NaOCl) & mercuric chloride ($HgCl_2$). (1994 kanyad *et al*., 1997: venkatchalam *et al*., 1999 Sharma & anjaiah 2000.) 1 % Sodium hypochlorite for 5-10 minutes was found better than mercuric chloride for controlling the infection and it had not any adverse effect on explants even long duration. (Miller and lipschutz,1984; Naik and Chandra, 1993 and Villafranca, 1998). Gopal *et al*. (1998) have reported the use of mercuric chloride for 5 minutes. Therefore the objective of the work is plant propagated through explants (seeds, cotyledon, and shoot tip) using Invitro technique. *Cassia tora* can easily be grown from seed, but due to their hard seed coat the germination appears to be a limiting factor; many leguminous seeds have wide dormancy duration induced by the appearance of a hard water proof coat. Among breaking dormancy the most widely used pre-germination treatment. (Rolestan M.P.) (1978), Ballard LAT, (1973). Cavanagh (1980), Following by which breaking dormancy treatment of other cassia species available but *cassia tora* is inadequate. Most commonly Invitro propogated Bahunia plants are derived from Invitro seed germination & (singh *et al*.2012) provide a simple but apparently effective protocol for seed sterilization for to germination Invitro. Although more difficult Bahunia cultures can be also initiated from mature plants (kumar 1992, Mathur & mukuntha, dhar & upreti 1999, kumar 2005) seed are three variety Roma, Riogarande & Moncy maker were treated with different concentration of clorax (sodium Hypochloride) to optimize level of clorax suitable for Invitro germination. However plantlets could be micro-propogated from nodal & shoot tip explants of obtained from seedlings (yadav *et al*.1990) Therefore the present investigation was undertaken to develop a protocol for the Invitro propogation & to compare the phytochemical analysis between Invitro propogated plants ref.callus proliferation of tomato cultivars. Seeds were inoculated on MS medium for Invitro germination. However plantlets

MATERIAL & METHOD

Collection of plant material: *Bahunia purpurea, cassia tora, Syzygium cumini, L.* Three plants were collected from Dr. B.A.M.U. Sub campus area.

Surface Sterilization procedure: seed, Shoot tip removed from pod washed with using sterile D/W add drop of mediclore for 20 min to remove dust particle etc. Then given treatment with 0.1 % bovistin (antifungal solution) to avoid fungal infection, after half an hour all these seeds were taken in laminar air flow for further sterilization. Omit out fungicide solution & wash using sterilized D/W having a drops 10% teepol / tween 20 for 2 min. of to remove all dust particles & antifungal remants for three to four times.

Taken Gibberlic Acid (GA3) to improve the Invitro seed germination minimum on the half Strength M.S, media with various concentration 5 mg to 10 mg/ppm/liter, add in seeds kept for half an hour Treat these seeds & shoot tip using 70% alcohol for 45 second or 60 second & finally with 0.1 to 2 % $Hgcl_2$ % & 6% $Nacl_2$ for 2-3 min, 3-4 times with sterile D/W.

Preparation of explants & Inoculation: Split the seed into two halves to expose embryo (To enhance the germination percent) Inoculate the embryo Containing cotyledon halves on half strength M.S. medium for seed germination.

Incubation of seed germination: incubate cultures in dark for 8-days & then given 8: 16 light & dark photo cycle for 15 to 20 days.

Media*:* (gamborg ., Ishag 2009, Khalafalla 2010)
The medium needed for development of material for Invitro studies. (Murashige & Skoog 1962) The Stock includes Ms Major, MS minor, Fe-EDTA, Inositol, vitamins & hormne.

Half M.S. Media: the seeds were inoculated on half strength MS medium (without sucrose, & Growth Hormone.)

Full M.S. Media: with Sucrose & Growth Hormone

Culture Condition: The growth room temperature maintained for Invitro cultures were (25+- 2) 60 to 70 % relative humidity the light intensity was given 12-12, 10-14, & 8-16 hours for growth of seed germination & 8-16 photoperiod was given to growing transfer plant. The data was analyzed for seed germination percentage, non germination of seeds.

Effect of plant growth regulator on Plant regeneration: A low concentration of auxin (2-4 D NAA) used for root developed & high concentration of auxin calli formation (link & eggers 1998), higher concentration cytokinin (0.1 to 10mg/L) induced adventitious shoot formation & inhibit root formation. (baraun 1998) Dormancy can be overcome by treating the dormant seeds with GA3 & cytokines (Bose TK., 1986, Ruth R.F.1987)

Extraction: Take Invitro propagated plant crush with mortal pestle to make fine powder, Dissolve 1% in 10 ml methanol (organic Solvent) & Aqueous. Kept overnight for Shaker after 24 hours, It was filtered residue was discarded.

Phytochemical Analysis: Qualitative analysis was done by the presence Phenolic, Flavonoids, Tannins, Indole Alkaloids, Saponin, Triterpenoids, Reducing sugar (Azra Kamal -2014, Manmuthu Krishnaveni 2015, Kokate C.K. *et al*.2001)

Saponins: About 0.5 ml of extract was taken with 5ml D/W & then heated to boil frothing (appearance of creamish of small bubbles) shows the presence of saponins.

Tannins

Ferric Chloride test: 2-3 ml extract was taken in test tube & 10% $fecl_3$ (ferric chloride solution) 0.5ml was added into it, Dark blue or greenish grey coloration was observed compare with negative test. This is confirmation test for tannin.

Flavonoid

Alkaline reagent test: Methanol extract add few drop of sodium Hydroxide 10% (NaOH) +2-3 drops dilute Hydrochloric acid were added in test tube. Yellow solution turned colorless on addition of dilute Hcl which indicate Positive test. Secondly aqueous extract was added in 10% $Fecl_3$, a green precipitate indicated positive test.

Cardiac Glycosides: Methanol extract (2 ml) + 3.5 % of $Fecl_3$ (0.5ml) +0.5 ml Glacial acetic acid + 2ml of conc. H_2SO_4 was taken in beaker, reddish brown ring at interphase is indicator of positive test.

Steroids & Terpenoids: Methanol extract (1ml) + 1ml Chloroform + 2-3 ml acetic anhydride & 1-2 drops of conc. H_2SO_4 were added. Dark green coloration of the solution indicates the presence of steroids & pink or red coloration of the solution indicates that presence terpenoids.

Salkowshi test: 2ml of concentrated sulfuric acid was added to the extract, a yellow ring was formed at the junction, which turned red after one minute.

Indole Alkoloids: Methanol extract + Conc. $H2SO_4$ + Potassium dichromate was taken in flask color change is confirmation for presence of Indole Alkaloids.

Wagner's test (Solution of iodine in potassium iodide): Alkaloids give reddish brown precipitate with Wagner's reagent. (Mix 1.27 g Iodine with 2g potassium iodide and make it up to 100 ml)

Reducing Sugar: Methanolic extract + 5 ml of equal volume of Fehling Solution A & B boiled for 5 min. rusty brown. Precipitate is indication of positive test

Photos: a) Seed germination Stage of S.C., B.P., C.T. b) Regeneration Stage c) Multiplication Stage

Table Effect of half strength Media on seed germination percentage & node with different photoperiod.

Plant Name(Explant)	Media	% of seed Germination	Photoperiod (light-dark)	pH	Duration
B.P. (Seed)	H.S. Media	30%	12-12	5.8	
		50%	10-14	5.8	
		70%	8-16	5.8	15-20 days

C.T.(Seed)	H.S. Media	30%	12-12	5.8	
		50%	10-14	5.8	
		70%	8-16	5.8	45days
S.C (Node)	Full M.S. Media	60%	12-12	5.8	30days

Table Invitro multiplication of regenerated plants

Plant Name	Media	Hormone	Hormone Concentration 1mg/1ml	photoperiod	PH	Duration
B.P	Full M.S.Media	BAP/IAA	2µl/1ml 0.5µl/1ml	8:16	5.8	25 days
S.C.	Full M.S.Media	BAP/IAA	2µl/1ml 0.5µl/1ml	8:16	5.8	30 days
C.T.	Full M.S.Media	BAP/IAA	4µl/1ml 0.5µl/1ml	8:16	5.8	40 days

Table Analysis of Chemical Compounds

Secondary Metabolism	*Syzygium Cumini*	*Cassia Tora*	*Bahunia purpurea*
Saponins	+	++M./+A	+M/++A
Tannin	++	++	++M/++A
Flavonoids	++ M./--A	++	++
Car, Glycosides	++	-	+
Steroids	++	+	+
Terpenoids	+M/+A	++	+
Red. Sugar	++	+	++
Ind.Alkaloid	++	+	+

Note: + present, ++ High activity, -- Absent, M.(Methanol), A(Aqueous)

RESULT & DISCUSSION

Plant secondary metabolites are unique source for pharmaceuticals food additives, flavors & other industrial material. (jian Zhaou 2005) Humans use secondary metabolites as medicines, flavorings & recreational drug in the recent past. (Srivastava et.al. 2007) Flavonoids present in high concentration in the epidermis of leaves & the skin of fruits & have essential role as UV protection, pigmentation stimulation of nitrogen fixing nodules & disease resistance (koes *et al* 1994: pierpoint 2000) Tannins binds to salivary proteins producing a taste which humans recognize as astringency (Clifford 1997). Plant saponins are widely distributed amongst plants & have a wide range of pharmacological properties (Estrada *et.al* 2000) such as anti-inflammatory (just 1998, sirtori 2000, li *et.al* 2002) Antifungal (sindombiwe 1998) Antibacterial (Killer et, al. 1998) antitumor (mimaki et, al. 2001) antioxidant (Huong *et,al*., 1998) activities. Terpens posses promising biological activities including antimicrobial activity (Dorman& deans 2000) antiviral activity (Schnitzler *et,al*. 2001) analgesic activity (Barocelli *et,al*. 2004) anti-inflammatory (Ambeoku *et,al*., 2001)The chemical Component in Syzygium Cumini present Saponins, tannins, flavonoids, cardiac Glycosides, Steroids & terpenoids, Reducing sugar, Indole Alkaloids. Tannins Flavonoids Cardiac Glycosides, Steroids Reducing Sugar Indole Alkaloids present in higher concentration flavonoids present in methanol extract absent in Aqueous. Terpenoids present in lower concentration Syzygium cumini leaf. Saponins Tannin, Terpenoids present higher concentration & Steroids Reducing Sugar, Indole Alkoloids, low Concentration in *cassia tora*. Saponins, Cardiac Glycosides, Reducing Sugar, Flavonoids, Indole Alkaloids present higher concentration, tannin, flavonoids, steroids, terpenoids, Indole Alkalods in *Bahunia purpurea*.

Bahunia purpurea, *Cassia tora* seeds suitable for germination on half strength media with proper growth regulators (Auxin & cytokinin 1mg/ml) & temperature. The seed germination percentages results obtained 30.50,60 & 70 % with given different photoperiods 12-12, 10-14,8-16. In that germination of *Bahunia Purpurea* rate is highest of 70 % after 8-16 photoperiod, germination occur between 15-20 days, like that given same percentages & photoperiode to *cassia tora* seed germination found within 45 days, lastly nodes as (explant) of *Syzygium cumini* growth shows 60% with 12-12 photoperiode within 30 days, media pH was same 5.8-6. Invitro *Bahunia purpurea, Cassia tora* & explants (node) of *Syzygium cumini* used for shoot proliferation, on full MS media, maintain media pH 5.8 upto 6 with hormone concentration of BAP/ IAA (2µl/ml, 0.5µl/ml) for *bahunia purpurea, Syzygium cumini & cassia tora,* taken higher concentration of cytokinin & auxin was same (4µl/ml, 0.5µl/ml), in the multiplication stage *Bahunia purpurea* plant shows earlier response within 25 days, *Syzygium cumini* within 30 days & finally *Cassia Tora* growth found late responses within 40 days.

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