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Investigation of Bioactive Compounds of *Capsicum Frutescence* and *Annona Muricata* by Chromatographic Techniques

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ABSTRACT

Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. The focus of this paper is on the analytical methodologies, which include the extraction, characterization of active ingredients in *Capsicum frutescens* fruits and *Annona muricata* L. leaves. *Capsicum frutescens* used for various problems with digestion including upset for conditions of the heart and blood vessels including poor circulation, excessive blood clotting, high cholesterol, and preventing heart disease. Whereas *Annona muricata* L. leaves are traditionally used to treat diabetes. People have been consuming raw leaves of *Annona muricata* L. to control blood glucose levels. The present investigation was designed to study the phytochemical profiling and bioactive component principles of *Capsicum frutescens* and *Annona muricata* by Thin layer Chromatography. Bio autography agar overlay test was done to detect the antimicrobial activity of the extracts. The results of this study confirmed the presence of various bioactive compounds in the acetone and methanol extracts of both the plants. The chromatographic analysis revealed that *Capsicum frutescens* fruit extract and *Annona muricata* leaf extract are composed of various Alkaloids, Terpenoids, Saponins, and Phenolics which are accountable for many biological activities. Bio autography assays shows that *Capsicum frutescens* shows growth inhibition against bacteria, but *Annona muricata* does not shown any significant activity. The findings of present study implies that both extracts are potent source for some medicinally important phytochemicals with antioxidant and antibacterial activities.

Keywords: Phytochemicals; Thin Layer Chromatography; Bio autography.

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INTRODUCTION:

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization [1]. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser cost [2, 3].

The *Capsicum* genus is also called hot pepper in the family Solanaceae comprises five domesticated species, *Capsicum chinense Jacq., C. annuum L., C. frutescens L., C. pubescens R. et P.,* and *C. baccatum L.* Even though there are various wild species, *C. frutescens L.*is one of the most known species in the genus with morphological characteristics, microsatellite (or simple sequence repeat - SSR) molecular markers and unique chloroplast genome. However solely two morphological types are widely cultivated. Hot peppers are grown by small-, medium-, and large-scale farmers, the cultivation fitting the small farmer models, as an alternative crop considered an important source of income to attend the fresh and the processed markets[4,5,6].

Capsicum frutescens contain *Burkholderia strains* bacterial strains, which are vital in remarkable induction of early flowering and fruiting along with protection from insect attack[7]. *Capsicum frutescens* used in Production of vanillin by bioengineering, ferulic acid is precursor of vanillin were found to be the intermediates in the phenylpropanoid

biosynthetic pathway of Capsicum species[8]. Ethylene positively regulates the hot pepper fruit colouration, while inhibition of Abscisic acid biosynthesis promotes colouration and de-greening[9]. *Capsicum frutescens* is not only an edible vegetable, but also used in folk remedies to inhibit the growth of gastric pathogen Helicobacter pylori, inhibit platelet aggregation, anti-diabetic, Gastrointestinal stimulant and potential anti-oxidant[10]. The acetonitrile extract of the seeds, peel and whole fruits contained antioxidant and antimicrobial activity[11]. It also possess a pesticide activity, when tested on *Rhipicephalus microplus* shown to lower egg production and hatching rate[12]. Hy-line Brown laying hens treated with hot pepper powder for 14 days, improved the yolk quality and egg weight[13]. Aqueous extracts of *C. frutescens* have potential for the control of *ichthyophthiriasis* as ectoparasite in the aquaculture industry, under test condition, it could kill the 70% of parasites post 4h administration without any acute toxicity on goldfish[14]. Hot pepper as a vital components of homeopathic medicine given to women for two weeks reduced menopausal hot flashes significantly[15]. Increased serum total cholesterol, triglycerides, high-density lipoprotein. and low-density lipoprotein caused by X-100 in rats reversed post treatment with *C. frutescens* 50-200mg/kg. Reduction in Triton X-100-mediated decrease in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose 6-phospahte dehydrogenase significantly reversed by the extract[16]. Free radicals reactions cause damage through oxidation process to membrane and disruption of metabolic pathways thereby increasing mutations in DNA and alteration of platelet function. Numerous studies have suggested that eating foods rich in phytochemicals and antioxidants has been linked with lessened risk of certain forms of cancer, stroke, and cardiovascular diseases[17]. Change in flavor from fruity to a more citrus-like aroma in red stage, thought to increase antioxidant capacity like oxygen radical absorbance capacity (ORAC) and the diphenylpicrylhydrazyl (DPPH) free radical method[18].

Annona muricata (*A. muricata*) is a tropical plant species belonging to family *Annonaceae* and also known as Graviola. The medicinal uses of the Annonaceae family were reported long time ago and since then, this species has attracted the attention due to its bioactivity and traditional uses[19]. Lead compounds from endophytic fungi associated with *Annona muricata* is responsible for the antimalarial medicinal properties[20]. Pharmacological studies conducted on 27 *Annona* species (*Annonaceae*) shown to have antiprotozoal, anti-tumoural, anti-diabetic, hepato-protective, anti-inflammatory and anxiolytic activities. The chemistry on the annonaceous acetogenins (ACGs) has been extensively investigated due to their potent anti-cancer activity[21,22]. *Annona muricata* crude extract inhibit the viability of breast cancer cells post 48 hours of treatment and promotes the apoptosis[23]. Ethanolic leaves extract of *Annona muricata* breast cancer histology and on proliferative indexes of DMBA-induced breast cancer rats[24].

MATERIALS AND METHODS

Collection and identification of plant material

The fruits of *Capsicum frutescens* were collected from Thrissur district of Kerala, India and leaves of *Annona muricata* were collected from the outskirts of Hosur, Krishnagiri district of Tamil Nadu, India. The type specimen was identified and authenticated by Dr. M. Kumar, Assistant Professor, Department of Plant Biology and Biotechnology, Madras Christian College, Chennai Tamil Nadu India. The same was deposited in the herbarium of PG and Research Centre in Biotechnology, MGR College, Hosur. The collected materials were cleaned shade dried and powdered for further extraction and analysis.

Preparation of Extracts

The shade dried plant material of *Capsicum frutescens* and *Annona muricata* were extracted in Soxhlet extractor for 8-10 hrs, with organic solvents viz., Methanol and Acetone in soxhlet apparatus for 10 hrs. After complete extraction, the extracts were filtered and concentrated under reduced pressure by using rotary vacuum evaporator. The extracts were dried in vacuum dryer and stored at room temperature until used.

Extract Recovery Percent

The extract recovery in different solvents was expressed as milligrams of the dry matter. The amount of crude extracts recovered after successive extraction were weighed and interpreted.

Thin Layer Chromatographic Studies:

TLC was performed on silica gel, G (Himedia, India) coated on the glass plate. Aliquots of extracts were spotted onto the TLC plate. TLC plates were spotted with methanol and acetone extracts.The spots were dried with a warm current of air and then the plates were developed in a saturated glass TLC tank using the appropriate solvent system. The solvent system used was Petroleum Ether: Ethyl acetate in 90:10 ratio. The TLC plates were placed in the solvent saturated TLC tanks, developed in the solvent system The chromatogram was visualized under UV-light (365nm) and different spray reagents. The Rf values of the coloured spots were recorded.

Detection of metabolites using thin layer chromatography

Identification of the compounds in the hot water, acetone and hexane extracts of the *Capsicum frutescens* and *Annona muricata* were performed on TLC plates that had been spotted with extract and allowed to develop. For each detection reagent, two identical plates were prepared alongside each other. The first plate was used as the reference whilst the second plate was subjected to spray reagents for detection. Methods for detection were performed as described by Wagner *et al.* (1984) and Krebs *et al.* (1969).

Detection of compounds was made by:

1. Exposure to Iodine vapor

2. Visualization under UV light (254 nm)

3. Lightly spraying the TLC plates with detection reagents and observing under visible light.

Phytochemical Screening:

Phytochemical screening was performed on TLC plates that had been spotted with extract and allowed to develop. Methods for detection were performed as described in Wagner et al.(1984) and Krebs et al.(1969).Detection of compounds was by :1) visualization under visible or UV light (365nm), lightly spraying the TLC plates with detection reagents and observing under visible or UV light.

Detection Reagents:

Vanillin Sulphuric Acid (vs)

The TLC plate was sprayed with 10ml of (A) followed by 5 to 10ml of (b), warmed at 100°C for 5 to 10 minutes and evaluated under visible light.

Liebermann-Burchard Reagent (LBr)

The TLC plate was sprayed with 5 to 10 ml of LBr reagent, warmed at 100°C for 5 to 10 minutes and evaluated under visible or UV (365nm) light.

Potassium hydroxide (KOH)

The TLC plates was sprayed with 10ml of a 10% (w/v) ethanolic KOH solution,dried and then observed under UV (365nm) or visible light, with or without warming.

Natural products-Polyethylene Glycol (NP-PEG)

NP-PEG was used for the detection of anthracene derivatives,coumarins,arbutin drugs,bitter principles and flavonoids,10ml of NP (1% (w/v) methanolic diphenylboryloxyethylamine) polyethyleneglycol-4000) was sprayed onto the TLC plate.The plate was then observed under UV light at 365nm.

Fast Blue Salt (FBS)

Fast blue salt was used for the detection of flavanoids and phenolic water,dried and then observed under visible light for the presence of red to brown zones, with or without warming.

Folin-Ciocaltue Reagent

Folin-Ciocalteu reagent was used for the detection of phenolic compounds and was purchased ready made from Merck(Darmstadt, Germany).The TLC plate was sprayed with 5 to 10ml and then evaluated in visible light for the presence of blue zones.

Dragendroff Reagent

Freshly prepared Dragendroff reagent was used for the detection of alkaloids.The reagent was prepared by dissolving 8g of KI in 20ml of H2O.This solution was then added to a second solution containing 0.85g of basic bismuth nitrate in 40ml of H2O and 10ml of acetic acid.The TLC plate was sprayed with 10ml and observed under visible light for the presence of yellow zones.

Aluminum chloride (AlCl3)

AlCl3 was used for the detection of flavanoids.A TLC plate was sprayed with 5 to 10 ml of a 1%(w/v) ethanolic AlCl3 solution and evaluated under UV (365) light.

Bioautography Agar Overlay:

Bioautography was performed using TLC plates that contained spots of interest. The TLC plates were cut to size and placed into petridishes.A volume of 12ml of molten agar, kept at 50°C,was inoculated with 200μl of on overnight bacterial culture,mixed thoroughly, and then poured over the TLC plate in petridish.The agar was spread uniformly over the plate to give an agar overlay thickness of approximately 1mm.The surface of the agar was flamed briefly to remove any air bubbles. Once the agar was set, the plates were inverted and incubated overnight at 37°C.The plates were sprayed with MTT (thiazolyl blue tetrazolium bromide) (2 mg /ml) and reincubated for 30 minutes at 37°C.The plates were then observed for spots that exhibited clear zones of growth inhibition against a purple background of live bacteria.

RESULTS AND DISCUSSION

Detection of components in the different extracts by thin layer chromatography

TLC plates were spotted with methanol and acetone extracts of both plants shown the presence of various active compounds.

Exposure to UV 254 nm and 365 nm

Several spots on the TLC plates showed faint purple or blue fluorescence in the presence of UV 365 nm light, shown in Figure 1. And also illustrated along with Rf values in Table 1 to Table 8. Acetone and Methanol extracts of *C. frutescens* acetone and methanol extracts showed eight spots with different Rf values Table1 and Table 3. Whereas Acetone and Methanol extracts of *A.muricata* showed ten and eight spots respectively, demonstrated in Table5 and Table 7.

Spray Reagent Detection of Active Components

Most of the compounds on TLC plate require visualization using appropriate spraying reagent.Spray reagent detection of active compounds in various extracts shown in Figure 2 and Figure3.Acetone and Methanol extracts of *Capsicum frutescens* are shown in table 1 & table 3 whereas for *Annona muricata* shown in 5+7.activity was seen except for NP-PEG, KOH and $AlCl_3$. *Annona muricata* acetone + methanol extracts.

Vanillin Sulphuric Acid (vs)

All active components exhibited colour violet in the visible light indicating the presence of terpenoids.The color reaction with this reagent is strong, suggesting that the active components were terpenoid type.

Liebermann-Burchard Reagent (LBr)

All active components exhibited colour brown in the visible light indicating the presence of terpenoids.The color reaction with this reagent is strong, suggesting that the active components were terpenoid type.

Potassium hydroxide (KOH)

With this detection reagent, there exhibited no color by the active components indicating they were not anthracene derivatives and coumarins.

Natural products-Polyethylene Glycol (NP-PEG)

There exhibited no color by the active components indicating the absence of anthracene.

Fast Blue Salt (FBS)

The appearance of red-brown zones by the active components indicated that this detection reagent can detect phenolic compounds.

Folin-Ciocaltue Reagent

The presence of blue spots after spraying with this detection reagent indicated the presence of phenolic compounds.

Dragendroff Reagent

The appearance of yellowish orange color after spraying with this detection reagent indicated the presence of alkaloids.

Aluminum chloride (AlCl3)

The active components did not react with this detection reagent indicating that they are neither coumarin nor flavonoid type compounds.

Detection of Classes of Compounds

TLC plates run in the mobile phase Petroleum ether: Ethyl acetate (90:10) resulted in the separation of nine components in the methanol and acetone extracts of

Capsicum frutescens and Annona muricata leaves. The spots were numbered from the origin. The spots 1, 2, 4,5and 7 were seen as Pink colour zones. Spots 3, 6 and fluoresced as blue colour at UV 365 nm and the spot 8 quenched fluorescence at UV 365nm.

The spot number 1 was pink colour with Rf value 13.3,14,7 and 13 respectively in methanol and extracts of *Capsicum frutescens and Annona muricata*. It tested positive for VS and LBr reagents indicating the prescence of terpenoids(Table2,4,9). It also tested positive for DR, Folin's, FBS reagent but tested negative for AlCl3 reagent, NP-PEG and KOH. This indicates the presence of Phenols and Alkaloids. (Table2,4,6,8)

The second spot was also pink in colour and had Rf values 20, 20, 13.3 and 20 respectively for methanol and acetone extracts. It dark brown in UV 254 nm. It tests positive for VS and LBr and turned blue when sprayed with Folin's reagent and turned yellow brown when sprayed with DR. The spot turned brown colour was observed in the spots at chromatogram when sprayed with Fast blue salt reagent. It tests negative for AlCl3, KOH and NP-PEG. Thus the component was identified for alkaloid and phenols(Table2,4,6,8).

The third spot with Rf value 33.3,33,20 and33 for methanol and acetone extracts appeared blue color in UV 365 nm. It test positive for VS and LBr reagent. The spot turned blue in the presence of Folin's and brown in the presence of fast blue salt reagent. It tests negative results when observed in AlCl3, KOH and NP-PEG reagent. This component was identified as terpenoids and alkaloids(Table2,4,6,8).

The spot number four had Rf value of 40, 40, 27 and 40 respectively for all methanol and acetone extracts, appeared pink in color and turned purple brown in response to for VS reagent. It turned brown in color after spraying with LBr reagent. It tested negative for NP-PEG, KOH and AlCl3 reagent. It tested positive for Folins reagent and FBS reagent. This compound may belong to the terpenoids and alkaloids group(Table2,4,6).

The fifth spot with Rf value 47, 47, 40 and 43 respectively for methanol and extracts appeared dull pink in UV 365 and reacted with Folin's reagent to give blue colour. The spot turned brown when sprayed with fast blue salt reagent. It also tested positive for DR, VS and LBr reagents. It tested negative for AlCl3, KOH and NP-PEG. This indicates that the components were alkaloids(Table2,4,6,8).

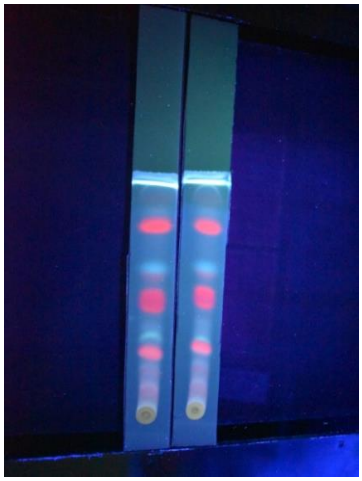
The sixth spot with Rf value 53.3,53,60 and 53for methanol and acetone extracts respectively appeared blue in UV (365 nm). It tested negative for AlCl3, NP-PEG and KOH. It tested positive showing yellow color formation when sprayed with DR, blue colour formation when the plates were sprayed with folin reagent and blue violet in visible light with VS reagent and brown when sprayed with FBS reagent. This was identified as alkaloids and terpenoids.

Spot number seven with Rf value 60, 60, 67 and 60 for methanol and acetone extracts respectively appeared light pink in colour under UV 365nm light. It tested negative for KOH and AlCl3 and also for NP-PEG. A blue colour was formed when the spot received folin reagent spray. The spot turned purple violet in colour when TLC plates were sprayed with VS reagent and a brown colour was observed for LBr reagent. This component was identified as a terpenoid with a phenolic group(Table2,4,6,8).

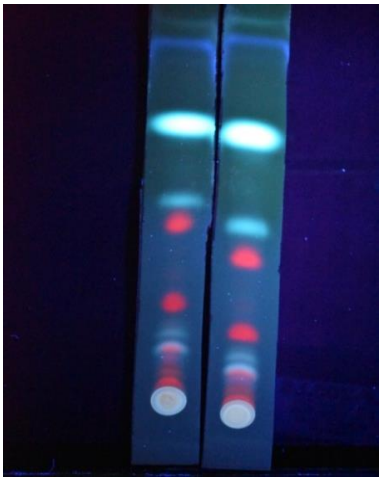
The eighth spot with 67, 67, 73 and 67 for acetone and methanol extracts respectively. It quenched fluorescence at UV 365 nm. It reacted with folin to give blue colour. It tested positive for VS, LBr and FBS reagent. It tested negative for AlCl3,NP-PEG and KOH reagents. The component in this spot has been identified as anthroquinone, alkaloids and terpenoids(Table2,4,6,8).

The spot number of nine and ten with Rf value 80 and 87 for acetone extract of *Annona muricata* quenched in UV 365nm. It tested positive for folin ,FBS,DR,VS and LBr by turning blue,brown,yellow, blue violet and brown in colour respectively. It tests negative after spraying with AlCl3,KOH and NP-PEG reagent. This component was identified as terpenoids and alkaloids(Table 6).

Figure 1 :Detection Of Components In Acetone And Methanolic Extracts Of *Capsicum Frutescens* And *Annona Muricata*



1



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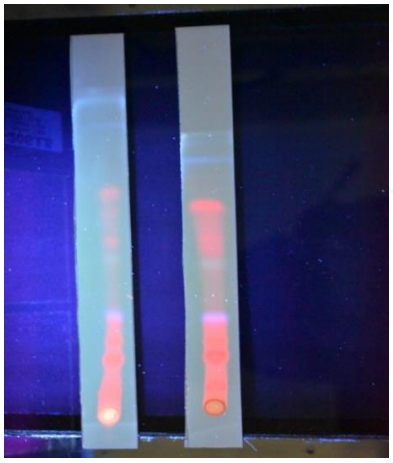

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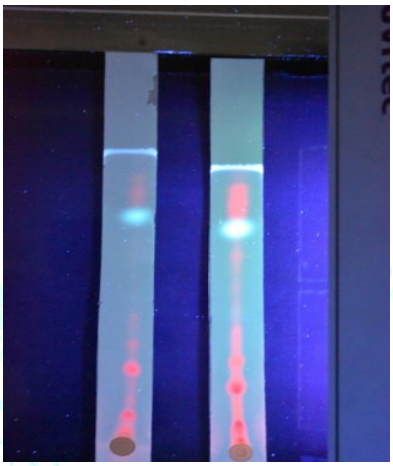


4

1.TLC Of Acetone Extract Of *Capsicum Frutescens*

2.TLC Of Methanol Extract Of *Capsicum Frutescens*

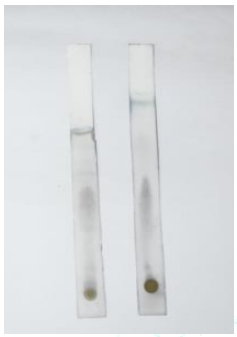
3.TLC Of Acetone Extract Of *Annona Muricata*

4.TLC Of Methanol Extract Of *Annona Muricata*

Figure 2 Spray reagent detection of active components in acetone and methanol extract of *Capsicum frutescens*


1


2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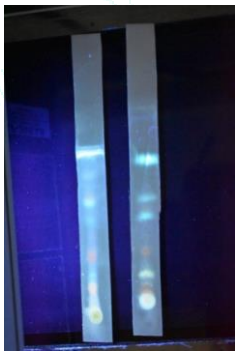
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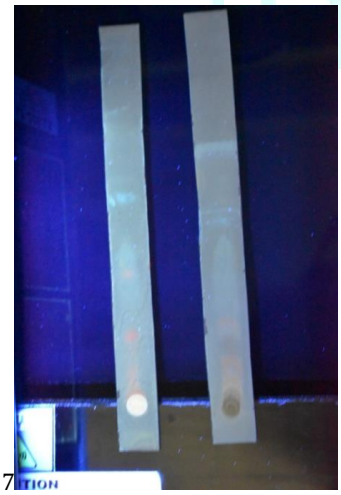
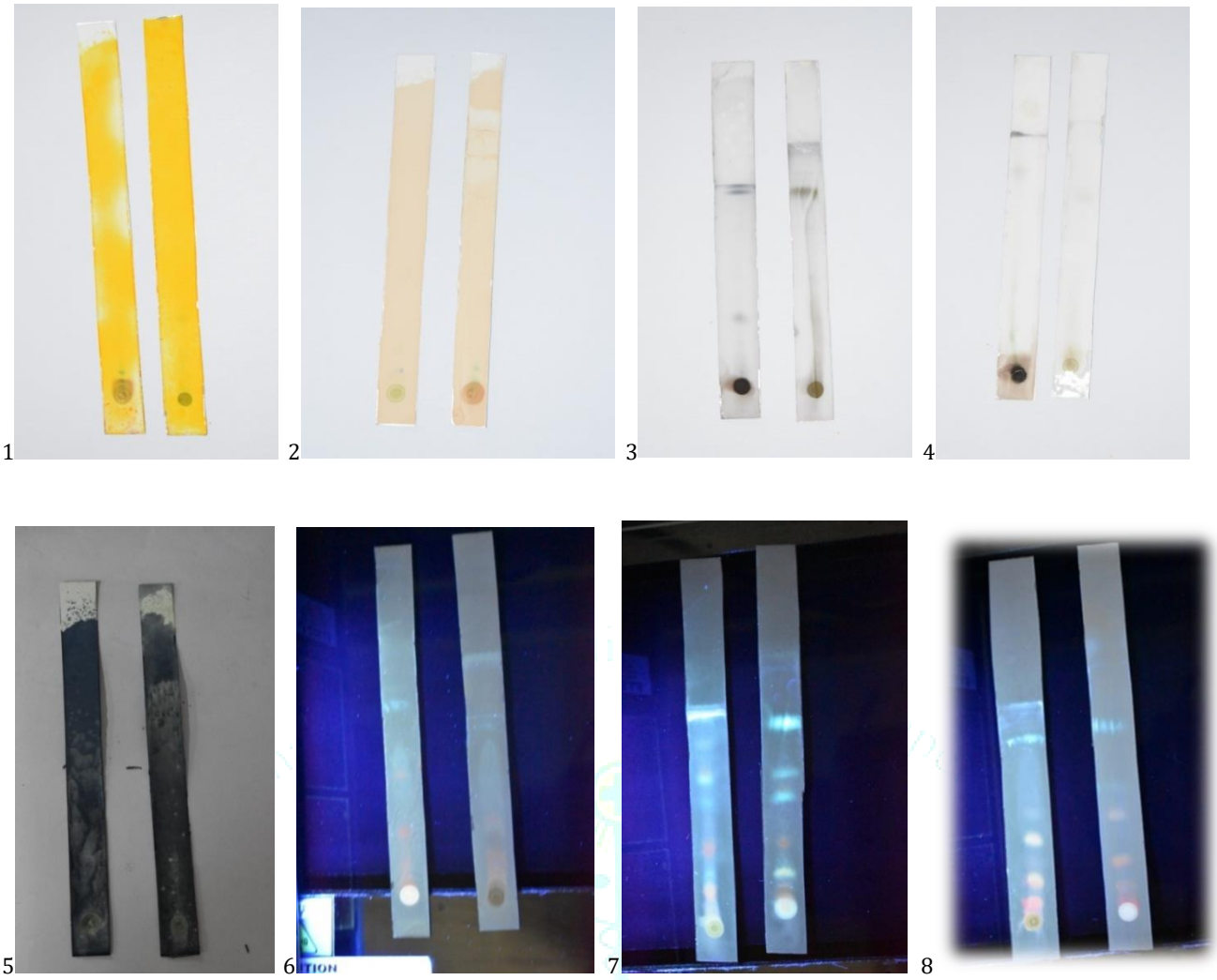
6


7


8

1.Dragendroff's test
2.Fast Blue Salt test
3.Vanillin Sulphuric Acid test
4.Libermann Burchard test
5.AlCl3 Test
6.KOH Test
7.NP-PEG Test
8.Fc-Reagent Test

Figure 3 Spray Reagent Detection of active components in *Annona muricata*



1.Dragendroff's test 2.Fast Blue Salt test 3.VanillinSulphuric Acid test 4.LibermannBurchard test

5.Fc-reagent test 6.KOH Test 7.NP-PEG Test 8.AlCl3 Test

Table 1 Spray Reagent Detection Of Active Components In Acetone Extract Of *Capsicum frutescens*

Identification of active components in the acetone extract of *Capsicum frutescens*								
Component	1	2	3	4	5	6	7	8
Rf x 100	13.3	20	33.3	40	47	53.3	60	67
UV-365nm	Pink	Blue	Pink	Light Pink	Light Blue	Pink	Pink	Q
VS	Violet	Violet	Violet	Violet	Violet Pink	Violet	Violet	Violet
LBr	Brown	Brown	Violet Brown	Brown	Brown	Brown	Brown	Brown
Folins	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
FBS	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
NP-PEG	-	-	-	-	-	-	-	-
KOH	-	-	-	-	-	-	-	-
AlCl3	-	-	-	-	-	-	-	-
Dragendroff	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

Components in the fractions were separated using the mobile phase Petroleum ether : Methanol (90:10); Q- quenching of fluorescence; Reaction colours noted were observed in visible light; * Colour observed at UV 365 nm;

Detection reagents:

DR, Dragendroff's reagent; VS, vanillin sulphuric acid reagent; LBr, Libermann-Burchard reagent; Folins, Folin Ciocalteau reagent; FBS, Fast blue salt reagent; KOH, Potassium hydroxide reagent; $AlCl_3$, Aluminium chloride reagent.

Table 2 Identification of Active Components in the Acetone Extract of *Capsicum frutescenes*

Compounds	Reagents	Detection	Identification
Alkaloids	Dragendroff's Reagent	Yellow Zones	+
Terpenoids	VS,LBr Reagents	Violet/Purple,Brown	+
Saponins	VS	Violet	+
Phenolics	Folin's Reagent	Blue Zones	+
Coumarins, Anthroquinones	KOH ,NP-PEG	Blue To Blue Violet Zones	_
Flavonoids	AlCl3	Bright Blue	_

Components in the fractions were separated using the mobile phase Petroleum ether : Ethyl actate (90:10);

+: Detected; -: Not detected.

Table 3 Spray reagent detection of active components in methanol extract of *Capsicum frutescens*

Identification of active components in the methanol extract of *Capsicum frutescens*								
Component	1	2	3	4	5	6	7	8
Rf x 100	14	20	33	40	47	53	60	67
UV-365nm	Pink	Blue	Pink	Light Pink	Light Blue	Pink	Pink	Q
VS	Violet	Violet	Violet	Violet	Violet Pink	Violet	Violet	Violet
LBr	Brown	Brown	Violet Brown	Brown	Brown	Brown	Brown	Brown
Folins	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
FBS	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
NP-PEG	-	-	-	-	-	-	-	-
KOH	-	-	-	-	-	-	-	-
AlCl3	-	-	-	-	-	-	-	-
Dragendroff	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

Components in the fractions were separated using the mobile phase Petroleum ether : Methanol (90:10);

Q, quenching of fluorescence; Reaction colours noted were observed in visible light; * Colour observed at UV 365 nm;

Detection reagents:

DR, Dragendroff's reagent; VS, vanillin sulphuric acid reagent; LBr, Libermann-Burchard reagent; Folins, Folin Ciocalteau reagent; FBS, Fast blue salt reagent; KOH, Potassium hydroxide reagent; $AlCl_3$, Aluminium chloride reagent.

Table 4 Identification of active components in the methanolic extract of *Capsicum frutescens*

Compounds	Reagents	Detection	Identification
Alkaloids	Dragendroff's Reagent	Yellow zones	+
Terpenoids	VS,Lbr Reagents	Violet/Purple,Brown	+
Saponins	VS	Violet	+
Phenolics	Folin's Reagent	Blue zones	+
Coumarins, Anthroquinones	KOH ,NP-PEG	Blue to Blue violet zones	_
Flavonoids	AlCl3	Bright blue	_

Components in the fractions were separated using the mobile phase Petroleum ether : Ethyl actate (90:10);

+: Detected; -: Not detected.

Table 5: Spray reagent detection of active component in the acetone extract of *Annona muricata*

Identification of active components in the acetone extract of *Annona muricata*										
Component	1	2	3	4	5	6	7	8	9	10
Rf x 100	7	13.3	20	27	40	60	67	73	80	87
UV-365nm	Pink	Blue	Pink	Light Pink	Light Blue	Pink	Pink	pink	Pink	Pink
VS	Violet	Violet	Violet	Violet	Violet Pink	Violet	Violet	Violet	Violet	Violet
LBr	Brown	Brown	Violet Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Folins	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
FBS	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
NP-PEG	-	-	-	-	-	-	-	-	-	-
KOH	-	-	-	-	-	-	-	-	-	-
AlCl3	-	-	-	-	-	-	-	-	-	-
Dragendroff	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

Components in the fractions were separated using the mobile phase Petroleum ether : Methanol (90:10);

Q, quenching of fluorescence; Reaction colours noted were observed in visible light; * Colour observed at UV 365 nm;

Detection reagents:

DR, Dragendroff's reagent; VS, vanillin sulphuric acid reagent; LBr, Libermann-Burchard reagent; Folins, Folin Ciocalteau reagent; FBS, Fast blue salt reagent; KOH, Potassium hydroxide reagent; $AlCl_3$, Aluminium chloride reagent.

Table 6 Identification of active components in the Acetone extract of A*nnona muricata*

Compounds	Reagents	Detection	Identification
Alkaloids	Dragendroff's Reagent	Yellow zones	**+**
Terpenoids	VS,Lbr Reagents	Violet/Purple,Brown	**+**
Saponins	VS	Violet	**+**
Phenolics	Folin's Reagent	Blue zones	**+**
Coumarins, Anthroquinones	KOH ,NP-PEG	Blue to Blue violet zones	_
Flavonoids	AlCl3	Bright blue	_

Components in the fractions were separated using the mobile phase Petroleum ether : Ethyl actate (90:10); +: Detected; -: Not detected

Table 7 Spray reagent detection of active components in the methanol extract of *Annona muricata*

Identification of active components in the methanol extract of *Annona muricata*								
Component	1	2	3	4	5	6	7	8
Rf x 100	13	20	33	40	43	53	60	67
UV-365nm	Pink	pink	Pink	Pink	Pink	Q	Q	Pink
VS	Violet	Violet	Violet	Violet	Violet Pink	Violet	Violet	Violet
LBr	Brown	Brown	Violet Brown	Brown	Brown	Brown	Brown	Brown
Folins	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
FBS	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
NP-PEG	-	-	-	-	-	-	-	-
KOH	-	-	-	-	-	-	-	-
AlCl3	-	-	-	-	-	-	-	-
Dragendroff	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

Components in the fractions were separated using the mobile phase Petroleum ether : Methanol (90:10); Q, quenching of fluorescence; Reaction colours noted were observed in visible light; * Colour observed at UV 365 nm;

Detection reagents:

DR, Dragendroff's reagent; VS, vanillin sulphuric acid reagent; LBr, Libermann-Burchard reagent; Folins, Folin Ciocalteau reagent; FBS, Fast blue salt reagent; KOH, Potassium hydroxide reagent; $AlCl_3$, Aluminium chloride reagent.

Table 8 Identification of active components in the methanolic extract of *Annona muricata*

Compounds	Reagents	Detection	Identification
Alkaloids	Dragendroff's Reagent	Yellow zones	**+**
Terpenoids	VS,Lbr Reagents	Violet/Purple,Brown	**+**
Saponins	VS	Violet	**+**
Phenolics	Folin's Reagent	Blue zones	**+**
Coumarins, Anthroquinones	KOH ,NP-PEG	Blue to Blue violet zones	_
Flavonoids	AlCl3	Bright blue	_

Components in the fractions were separated using the mobile phase Petroleum ether : Ethyl actate (90:10); +: Detected; -: Not detected.

Bio autography Agar Overlay:

In Bio autography assay, the antibacterial activity of the compounds separated on Thin layer chromatography was determined.

Actvity in *Capsicum frutescens*

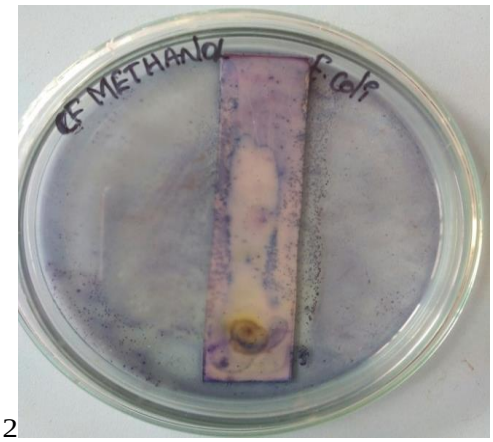
Agar overlay bio autography of the isolated compounds are demonstrated for the fractions with Rf values of 0.16,0.20,0.23,0.33,0.36,0.40, 0.52,0.66 in all the plates , showing inhibitory activities against gram bacteria *E.coli*. After incubation,zones of growth inhibition were observed.

Activity in *Annona muricata*

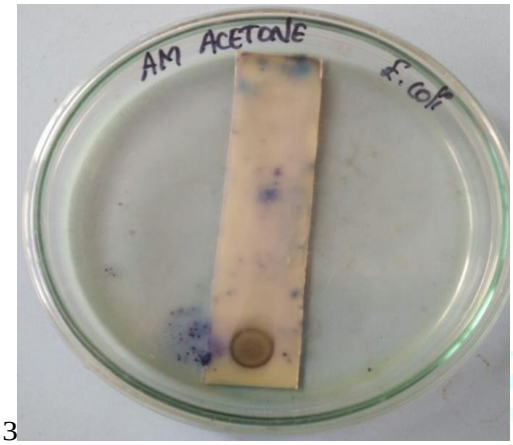
After incubation, the zones were not formed clearly indicating that there is no inhibition.

Figure 4 Bioautography

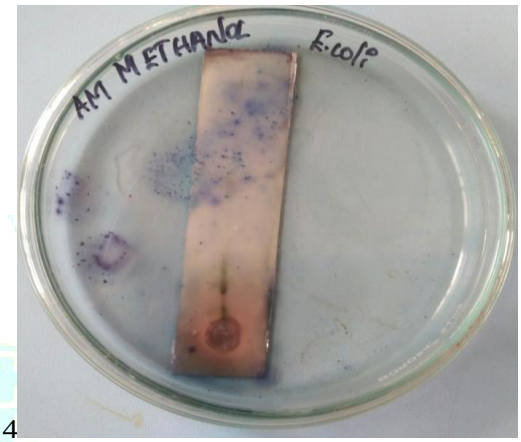
1



2



3



4

1.*Capsicumfrutescens* Acetone extract

2.*Capsicumfrutescens* Methanol extract

3.*Annonamuricata* Acetone extract

4.*Annonamuricata* Methanol extract

CONCLUSION

TLC is a fast and cheap method often used for the plant extract screening. The method is especially useful when combined with bioautography. The results of this study confirmed the presence of various bioactive compounds in the acetone and methanol extracts of both the plants. The chromatographic analysis of *Capsicum frutescens* fruit extract and *Annona muricata* leaf extract are composed of various Alkaloids, Terpenoids, Saponins, and Phenolics which are accountable for many biological activities. Bio autography assays shows that *Capsicum frutescens* shows growth inhibition against bacteria. The resulting study implies that both extracts possess medical applications because of the existence of antioxidant and antibacterial activities.

CONFLICTS OF INTEREST: Nil

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