Int.J.Curr.Microbiol.App.Sci (2016) *5*(10): 109-117

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 10 (2016) pp. 109-117**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.510.013

Preliminary Phytochemical Screening and Anti angiogenesis Studies of Different Extracts of *Curcuma amada*

A.L. Tariq[1]*

[1]Department of Biotechnology, Govt Degree College Beerwah-193411, Kashmir, India
**Corresponding author*

A B S T R A C T

The present study aimed for the identification of *Curcuma amada* in terms of biological activity with special emphasis to Anti-angiogenesis and anti cancer activity. The preliminary screening of the plant revealed the presence of different class of compounds in the rhizome extracts of *Curcuma amada*. These phytochemical compounds were identified on the basis of the screening tests showed the presence of the alkaloids, glycosides and anthraquinones. The precipitation test showed the presence of gum and mucilage in moderate quantitiy. The major quantity of active compounds was alkaloids while minor quantity of glycosides and anthraquinones was found. The rhizome extracts of *Curcuma amada* showed the presence of the phytochemical compound such as carbohydrates, phenols, flavonoids, tannins, steroids, terpenoids, saponins while absence of phenols, amino acids, oils and fats. The research studies showed that the crude form of rhizome *Curcuma amada* had more activity than peterolium ether extract, methanol extract and chloroform extract of rhizome *Curcuma amada*. The fertilized egg inoculation signified to identify which extracts of rhizome *Curcuma amada* have more activity. From this study it was identified that the crude form rhizome *Curcuma amada* had more anti-angioganesis activity than the peterolium ether, methanol and chloroform extracts. The anti-angiogenesis activities of drug 4-hydroxy benzaldehydes on the fertilized eggs were compared with the anti-angiogenesis activity of *Curcuma amada* in the fertilized egg. The in-vitro study supports the in-vivo studies. MTT assay in-vitro study reveals an anti-cancer activity of the crude form. The work reveals that the plant is a highly biological potent against cancer and further phytochemical investigation is to be initiated.

Keywords

Biological activity, *Curcuma amada*, Anti-angiogenesis, 4-Hydroxy Benzaldehyde.

Article Info

Accepted:
12 September 2016
Available Online:
10 October 2016

Introduction

Awareness of medicinal plant usage is a result of the many years of struggles against illnesses due to which men learned to pursue drugs in barks, seeds, fruit bodies and other parts of the plants (Tapsell *et al*., (2006).

Curcuma amada is medicinal plant which is used in several traditional medicines to cure various diseases. *Curcuma amada* has been shown to possess anti-inflammatory, antioxidant and antitumor properties (Ankita

and Chauhan, 2013). *Curcuma amada* Roxb is a unique spice having morphological resemblance to ginger but imparts raw mango flavor. The biological activities of mango ginger include antioxidant activity, antibacterial activity, antifungal activity, anti-inflammatory activity, platelet aggregation inhibitory activity, cytotoxicity, antiallergic activity, hypotriglyceridemic activity, brine-shrimp lethal activity, enterokinase inhibitory activity, CNS depressant and analgesic activity (Policegoudra *et al*., 2011). More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat the disease (Akram *et al*., 2010). Curcumin (diferuloyl methane), a small-molecular weight compound isolated from the roots of Curcuma longa L. (family Zingiberaceae), has been used traditionally for centuries in Asia for medicinal, culinary and other purposes (Badreldin *et al*.**,** 2006). Mangiferin, a bioactive compound having potent nutraceutical, strong antioxidant and pharmacological significance has been extracted using microwave-assisted extraction (MAE) technique from CURCUMA AMADA, commonly known as mango ginger (Jeke *et al*., 2013; Padmapriya *et al*., 2012). The phenolic content was the highest in methanol extract, followed by acetone, ethyl acetate and water extracts. Pharmacologist, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases (Carrubba *et al*., 2012). Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer. Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular disease. The sprouting angiogenesis occurs at a rate of several millimeters per day, and enables new vessels to grow across gaps in the vasculature (Shashank *et al*., 2013). Anti-angiogenesis drugs don't attack cancer cells directly, instead they target the blood vessels the cancer cells need to survive and grow. By doing this, they may help prevent new tumors from growing. They may also make large tumors shrink if their blood supply is cut off (Kerbel *et al.,* 2004). The angiogenic growth of blood vessels and lymphatic vessels coordinates several biological processes such as cell proliferation, guided migration, differentiation and cell–cell communication (Ralf *et al*., 2007). Angiogenesis, the formation of new blood vessels, is an integral part of both normal developmental processes and numerous pathologies, ranging from tumor growth and metastasis to inflammation and ocular disease (Auerbach *et al***.,** *2003).* The growth of new blood vessels from pre-existing vascular elements, or angiogenesis, involves coordinated signals to the adhesion, migration, and survival machinery within the target endothelial cell (Chris *et al*.**,** (2005).

Materials and Methods

Collection of the plant

The plant materials were collected from the market of Srinagar and taxonomically authenticated from the Deparment of Botany Govt Degree College Beerwah Kashmir. The collected plant material was then washed thoroughly in running tap water followed by distilled water. It was then dried at room temperature and powdered using mortar and pestle and subjected for extraction.

Extraction of plant material

The plant materials were extracted by adopting the Mandal *et al*., (2008)

Crude Extraction

Known quantities of the powdered rhizome material were de-fatted with n-hexane and then extracted with methanol, petroleum ether and chloroform by using soxhlet apparatus for 24 hrs (10 cycles). The extract was collected and solvent was evaporated to dryness at constant temperature of 65 °C at reduced pressure. The residues were weighed and stored at room temperature for further studies of phytochemical analysis and angiogenesis.

Preliminary Phytochemical Screening of the Plant

The methanolic rhizome extract of *Curcuma amada* was used for testing preliminary phytochemical screening in order to detect major chemical groups (Sahu and Saxena, 2012).

Test for carbohydrates

Molisch's test: 3 ml of Molisch's reagent was added to the 3 ml of *Curcuma amada* extract solution and gently shaked for few minutes. Then 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. The development of a purple ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's test: 1ml of Fehling's A and 1ml of B solutions were added to the test tube and boiled for 1 min. To this 2 ml of *Curcuma amada* etract was added and heated in boiling water bath for 10 minutes. Appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

Test for Flavanoids

Shinoda test: To 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added. A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent yellow precipitate indicated the presence of flavonoids.

Test for Tannins

Braemer's test: To 3ml of extract, 1 ml of 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for Steroid/Terpenoid

Liebermann-Burchard test: To 1ml of extract, 1ml of chloroform, 3ml of acetic anhydride and 2 drops of concentrated Sulphuric acid are added. The formation of dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for Alkaloids

Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.

Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.

Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

Legal's test: Dissolved the 0.1g of extract in 2ml pyridine followed by addition of 2ml of sodium nitroprusside solution and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

Foam test: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously for 20 minutes then some drops of olive oil were added. The formation of stable 1cm foam was taken as an indication for the presence of saponins.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

Ninhydrin test: 5 drops of 5% lead acetate solution was added to 5ml of *Curcuma amada* extract solution and were boiled on water bath for 10 min. After boiling the change in the colour of solution to purple or blue indicates the presence of amino acids.

Test for fixed Oils and Fats

Small quantity of the petroleum ether extract was pressed between two filter paper. The oil stains on the paper indicated the presence of fixed oils.

Note: the results for the above all experiments can be noted as If the response to the test is high it can be noted as +++which indicates that the particular group is present as the major class. If the response is average then note it as ++ indicates the presence in moderate quantity. If the response is very small then note it as + indicating the presence of only in traces. If no response is then negative.

Anti Angiogenesis Studies

An Anti-angiogenesis studies was carried out by following the methodology of Chris *et al*., (2005) taking the fertilizing egg in triplicates for the assay. Positive control were used as 4-hydroxy benzaldehyde.The extracts were introduced into the eggs using micro syringe.The eggs were then subjected to incubation for 15 days. After 15 days the eggs were opened and observed for Anti-angiogenesis.

MTT Assay (HeLa cell Line)

MTT assay was carried out by following Ankita *et al*., (2013). For adherent cells, the medium was removed and replace it with 100 µL of fresh culture medium. For non-adherent cells, microplate was centrifuged and pellet clees removed, carefully remove as much as possible and replaced it with 100 µL of fresh medium. 10 µL of the 12 mM MTT stock solution to each well was added. Including a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone and incubated at 37°C for 4

hours. At high cell densities (>100,000 cells per well) the incubation time was shortened to 2 hours. 100 µL of the SDS-HCl solution to each well was added and mixed thoroughly using the pipette. The microplate incubated at 37°C for 4– hours in a humidified chamber. The samples were mixed again and again to avoid decrease in the sensitivity of the assay. The absorbance values were read at 570 nm.

Results and Discussion

The taxonomical hierarchy of mango ginger is belonging to Kingdom: Plantae, Order: Zingiberales, Family: Zingiberaceae, Genus: Curcuma and Species: C. amada

Preliminary phytochemical screening

Preliminary Phytochemical studies revealed the presence of class of compounds present in the sample. The alkaloids were present in major quantity in the plant extract. The result shown in Table 1

Anti angiogenesis studies

Anti angiogenesis studies were carried out in dried powder of *Curcuma amada*. Extracts were prepared in methanol, chloroform and petroleum ether applied in each set of fertilized egg. The study revealed that the sample extracts were capable of attenuating the formation of blood vessels (Figure 1) by showing significant activity when compared with drug 4-hydroxy benzaldehyde (Figure 2).

MTT Assay

In-vitro anti cancer studies was performed in the sample so as to identify the correct variety which posses more anti cancer activity (Table 2).

Table.1 Preliminary phytochemical screening of *Curcuma amada*

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	+ +
Phenols	Ferric chloride test	-
Flavanoids	Shinoda test Lead acetate test	+ +
Tannins	Braemers test	+
Sterioids/ Terpenoids	Liebermann- Burchardt test	+
Alkaloids	Draggendrof's test Hager test Wagners test	+++ ++ ++
Glycosides	Legal's test	+
Saponins	Foam test	+
Anthraquinones	Brontranger's test	+
Aminoacids	Ninhydrin test	-
Oil and fat	Spot test	-
Gum and mucilage	Precipitation test	++

Note: ---indicate not present, + In traces, ++ present in moderate amount and +++ more amount is present

Table.2 MTT assay for the *Curcuma amada* Sample

Curcuma amada Sample Concentration (mg/ml)	MTT assay % viability
Control	97.25%
50	83.25%
100	60.25%
150	55.26%
200	44.25%
250	40.12%

Fig.1

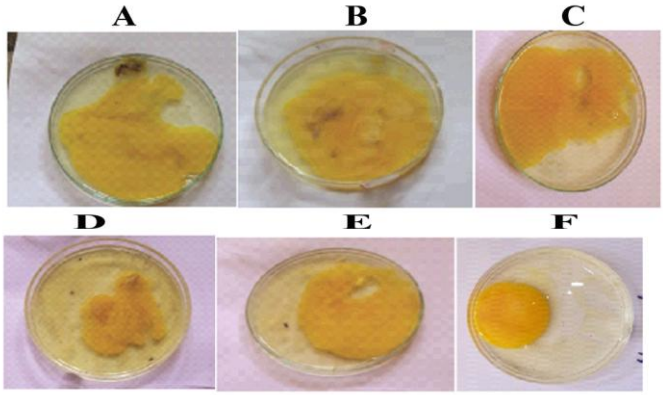

Figure 1. Anti-Angiogenesis studies on the fertilized eggs by using of 4-hydroxy benzaldehyde in differenet concentration viz (A) 50mg (B) 40 mg (C) 30 mg (D) 20 mg (E) 10 mg (F) Control without drug

Fig.2

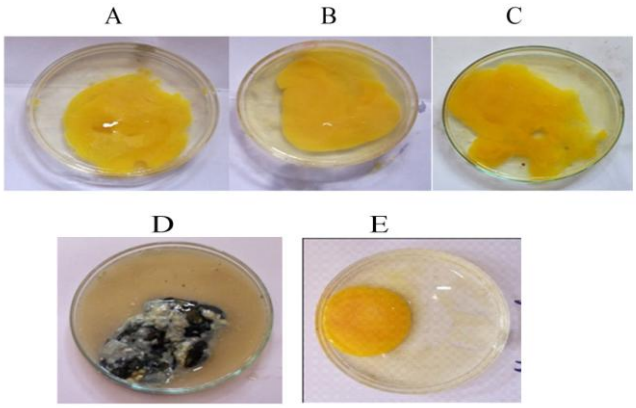

Figure 2. Anti-Angiogenesis study on the fertilized eggs by using of *Curcuma amada* extracts of (A) Petroleum Ether (B) Chloroform (C) Methanol (D) Crude (E) Control. .

Medicinal plants palys a vita role to cure many diseases which were not cured by the drugs due to reistance to them (Jha *et al*., 2013). The medicinal plants are preliminary screened for the phytochemical compounds that are present in the plant material (Sri *et al*., 2011). *Curcuma amada* used from the several traditions to cure various diseasesby showing anti-inflammatory, anti-tumor, anti-bacterial and anti-viral activities according to previous literature (Fatma and Arzu, 2013). In the present study the phtochemical analysis of *Curcuma amada* showed the absence of phenolic compounds while the presence of flavonoids, tanins, steroids, terpenoids, saponins, alkaloids, glycosides and anthraquiones in the extracts of *Curcuma amada* (Das *et al*., 2010). The ninhydrin test showed the absence of the aminao acids as there was no colour change in the test tubes containing the extracts of *Curcuma amada*. The oils and fats were completely absent in the extracts of *Curcuma amada* so does not have any role when compared with some species of zingiberaceace (Kathakali *et al.*, 2013). Angiogenesis is the process which plays an important role for the formation of tissue during developmental stages. There are some primary or secondary metabolic compounds found in many plant those could effect the angiogenesis process (Kerbel and Kamen, 2004; Prasath *et al*., 2013). In study on the extracts of *curcuma amada* in the fertilized eggs after 15 days of incubation was found that petroleum ether, chloroform extract and methanol extract does not showed any anti-angiogenesis activity thus revealed that blood vessel formation was normal (Sailendra *et al*., 2010). The crude extract of *Curcuma amada* which had altered the formation of blood vessel in the fertilized eggs thus showed anti-agiogenesis. The enrty of crude extract inside the parts of the fertilized eggs led the damage of blood vessels by causing the blood clot in the vessels. The drug which are used as anti-angiogenesis for the present invivo study 4 hydroxy benzaldehyde with different concentrations rangong from 10mg, 20mg, 30mg, 40mg and 50mg respectively to the fertilized eggs which showed anti-agiogenesis activity. The formations of the blood vessels in the fertilized eggs have been suppressed by the drug 4 hydroxy benzaldehyde (Amira *et al*., 2008). Cancer is more fatal disease an very sort are tried to control it by using anticaner medicines an alternative resources are traced from the plants which plays a duel role as being diet and medicinal values (Liu and Nair, 2012). In the present research anticancer activity of *Curcuma amada* have been studied by taking HeLa cell line derived from cervical cancer cells (Wasundara and Vasantha, 2013). The MTT assay of the *Curcuma amada* extract sample showed an anticancer acivity by inhiting cancer occoured in the cell line. The viability of the HeLa cancer cells showed decrease when there was an increase in the concentration of the *Curcuma amada* extract samples thus revealed have anticancer activity (Plengsuriyakarn *et al*., 2012; Tariq and Reyaz, 2012). So from the results it is cleared that the *Curcuma amada* have the ability to resist cancer and in future it may use as the drug to cure cancer.

In conclusion, from the present scientific investigation, we concluded that the *Curcuma amada* process significant anti-cancer activity in its crude form. Further studies on the crude material may lead to the identification of new anti –cancer compounds. From this it identifies that the crude form has more anti-angioganesis activity than the methanol, chloroform and petroleum ether extracts. The drug 4-hydroxy benzaldehyde used positive control to compare the activity with the *Curcuma amada*. The scope of the present work is to find out the biological activity of the plant

using in-vitro and in-vivo methods. The work reveals that the plant is a highly potent plant and further phytochemical investigation is to be initiated. Being a potent anti-cancer plant isolation of anti-cancer compounds from the plant is to be imitated.

References

Akram, M., Shahab-uddin, Afzal, A., Khan U., Hannan, A., Mohiuddin, E., and Asif, M. 1``2010. *Curcuma longa* and *Curcumin rom. J. Plant Biol.*,55(2): 65–70.

Alan Cantwell, M.D. 2010. Immortal HeLa cells and the continuing contamination of cancer and vaccine research- All Rights Reserved 2-17-10.

Amira, M.G.E., Amer, H., Helmy, W.A., Ragab, H.M., and Roba, M.T. 2007. Antiproliferative and Cancer-chemopreventive properties of sulfated glycosilated extract derived from *Leucaaena leucocephala*. *Indian J. Pharmaceutical Sci.,* 69(6): 805-811.

Ankita, J., and Chauhan, R.S. 2013. Phytochemical Analysis and Cytotoxicity Studies of *Curcuma amada* rhizomes in BHK-21 Cells. *Int. J. Scientific Res. Environ. Sci.,* 1(12): 365-371.

Auerbach, R., Lewis, R., Shinners, B., Kubai, L, Akhtar, N., Racchel, L., Brenda, S., Louis, K., and Nasim, A. 2003. Angiogenesis Assays: A Critical Overview. *Clin. Chem.*, 49(1): 32-40.

Badreldin, H.A., Husnia, M., Salwa, A., Noureldayem, C., Amel, O.B. and Gerald, B. 2006. Biological properties of curcumin: A review. 255 1(6): 509-521.

Carrubba, A., and Scalenghe, R. 2012. Scent of Mare Nostrum ― Medicinal and Aromatic Plants (MAPs) in Mediterranean soils. *J. Sci. Food and Agri.,* 92(6): 1150–1170.

Chris, S., David, M., and Dawyne, G. 2005. Stupack angiogenesis assays in the chick CAM cell migration. *Methods in Mol. Biol.,* 294: 123-136.

Das Kesari, V., and Rangan, L. 2010. Plant regeneration in *CURCUMA* species and assessment of genetic stability of regenerated plants. *Biologia Plantarum*, 54(3): 423-429.

Fatma, P.K., and Arzu, T. 2012. Biological screening of various medicinal plants extracts for antibacterial and antitumor activities. *Turk. J. Biol.,* 36: 641-65.

Jeke, K., Abhishek, D., Denis, C., Surbhi, C., and Debjani D. 2013. Experimental and modeling studies on microwave-assisted extraction of mangiferin from CURCUMA AMADA. *Biotechnol.,* 1: 100-125.

Jha, H., Anand, B., Mithlesh, P., Keshaw, R.A., and Sunil, S. 2013. Antimicrobial activity of rhizome of selected curcuma variety. *Int. J. Life Sci. Biotechnol. Pharamacy,* 2(3): 183-189.

Kathakali, B, Sarma, G.C., and Kalita, S. 2013. Antimicrobial efficacy of essencial oils extracted from some species of zingiberaceae. *Int. J. Appl. Biol. Pharma. Technol.,* 4(3): 110-118.

Kerbel, R.S. and Kamen, B.A. 2004. The anti-angiogenic basis of metronomic chemotherapy. *Nature Review Cancer,* 4: 423-436.

Liu, Y. and Nair, G.M. 2012. *Curcuma longa* and *Curcuma mangga* leaves exhibit functional food property. *Food Chem.,* 135: 634–640.

Mandal, V., Mohan, Y., and Hemalatha, S. 2008. Microwave assisted extraction of Curcumin by sample-solvent dual heating mechanism using taguchi L9 orthogonal design. *J. Pharma. Biomed. Anal.,* 46: 322-327.

Padmapriya, K., Abhishek, D., Surabhi, C. and Debjani, D. 2012. Microwaves assisted extraction of mangiferin from *Curcuma amada. Biotech.,* 2(1): 27-38.

Plengsuriyakarn, T., Viyanant, V., Eursitthichai, V., Tesana, S., Chaijaroenkul, W., Itharat, A., and Na-Bangchang, K. 2012. Cytotoxicity, Toxicity, and Anticancer Activity of *Zingiber officinale* Roscoe against Cholangiocarcinoma. 13(9): 4597-4606.

Policegoudra, R.S., Aradhya, S.M., and Singh, L. 2011. Mango ginger (*Curcuma amada* Roxb.) – A promising spice for phytochemicals and biological activities. *J. Biosci.,* 36(4): 739-748.

Prasath, D., Suraby, E.J., Karthika, K., Rosana, O.B., Prameela, T.P., and Anandaraj, M. 2013. Analysis of differentially expressed genes in CURCUMA AMADA and ZINGIBER OFFICINALE upon infection with RALSTONIA SOLANACEARUM by suppression subtractive hybridization. *Acta Physiologiae Plantarum,* 35(12): 3293-3301.

Ralf, H.A., and Kari, A. 2007. Molecular regulation of angiogenesis and lymphangiogenesis *Nature Reviews Mol. Cell Biol.,* 8: 464-478.

Sahu, R., and Saxena J. 2012. Evolution of phytochemical constituent in conventional and non-conventional species of curcuma. *Int. Res. J. Pharm.,* 3(8): 203-204.

Sailendra, S., Jonnala, K.K., Dharmendra, S., Karuna, S., Jay Prakash, T., Arvind, S.N., and Suchitra, B. 2010. A bioactive labdane diterpenoid from Curcuma amada and its semisyntheticanalogues as antitubercular agents. *European J. Med. Chem.,* 45: 4379-4382.

Shashank, M., Ajay, K.J., Manoj, J., Cathrin, M., and Debjit, B. 2013. Analgesic and Anti-Inflammatory Activityof *Kalanchoe Pinnata* (Lam.) Pers. *J. Med. Plants Studies,* 1(2): 24-28.

Sri, N.A., Guan, S.L., Sok, L.H., Hashim, Y., Norhanom, A.W., Faizal, W., and Syed, A.A. 2011. Phytochemical and Cytotoxic Investigations of Curcuma mangga Rhizomes. *Mol.,* 16: 4539-4548.

Tapsell, L.C., Hemphill, I. Cobiac, L., Sullivan, D.R., Fench, M., Patch, C.S., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A. and Inge, K.E. 2006. Health benefits of herbs and spices: the past, the present, the future. *Med. J. Australia,* 185(4): S1-S24.

Tariq, A.L., and Reyaz, A.L. 2012. Isolation of Cannabinoids from the Plant *Cannabis sativa* and its Potential anticancer Activity. *Int. J. Drug Develop. Res.,* 4(1): 241-246.

Wasundara, F., and Vasantha, R. 2013. Anticancer Properties of Phytochemicals Present in Medicinal Plants of North America. Using Old Solutions to New Problems. *Natural Drug Discovery in the 21st Century,* pp:159-180.

How to cite this article:

Tariq, A.L. 2016. Preliminary Phytochemical Screening and Anti angiogenesis Studies of Different Extracts of *Curcuma amada. Int.J.Curr.Microbiol.App.Sci.* 5(10): 109-117.
doi: http://dx.doi.org/10.20546/ijcmas.2016.510.013