Pharmacological Investigations of Methanolic Extract of *Mangifera indica* Peels

A project submitted

by

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Certification Statement

This is to certify that this project titled '**Pharmacological Investigations of Methanolic Extract of *Mangifera indica* Peels**' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Sabiha Chowdhury, Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

_____

Countersigned by the supervisor

_____

Acknowledgement

At first, I would like to thank Almighty Allah, to enable me to study in Pharmacy as well as to submit this project paper for the degree of Bachelor of Pharmacy, Department of Pharmacy, BRAC University, Dhaka.

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I am grateful to my father, mother, sister and friends for their immense mental support throughout my research work. Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

I am dedicating this paper to my beloved parents Wahidun Noor and Kaisara Akther Khanam and my grandfather Lt. Mubarak Ali Miya who always believed in me.

Abstract

Mangifera indica has many traditional uses int reating various diseases which has been scientifically proven by its antioxidant,immunomodulatory,anti-inflammatory,anti-allergic,anti-diabetic,antiviral,antibacterial,anti-fungal and monoamine-oxidase inhibitory effects.In this report powdered *Mangifera indica* peelwas extracted with methanol which was further screened for phytochemical analysis.Phytochemical screening revealed the presence of flavonoid, saponins, tannins, steroids, terpenoids, glycosides, alkaloids and carbohydrates in the peels of *Mangifera indica*. In the screening of hypoglycemic activity, Glibenclamide was used as a standard drug at a dose of 10mg/kg and methanolic extract of *Mangifera indica* was used a study sample. Methanolic crude extract at a dose of 200mg/kg and 400mg/kg showed moderate blood glucose lowering activity of 66.06% and 54.88% respectively after 90 minutes of oral administration of glucose.Methanolic extract was also evaluated for cytotoxic activity following brine shrimp cytotoxicity bioassay where Vincristine sulfate was used as standard and the study samples showed LC_{50} of 2.04 µg/ml.

List of Abbreviations

BSL Brine Shrimp Lethality

Me Methyl extract

CLT Controlled group

STD Standard group

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1.Introduction:

1.1 Conceptof medicinalchemistry:

Therapeutic Chemistry is the atomic investigation of medication, plan to investigate about putting upamedicationwiththenaturalcapacity andalsotheideaforsaletothepublicdrug's concoctionstructure.Itisa fieldof Pharmaceuticalscience inwhichthe procedure of blending newpharmaceuticalitemsconnectedtoaccomplishanoutcomethatwould promptttheprocessof developingofanothermedication.Itisa fortifyingfieldthattakesinto accountthejointeffort with different researchersto servethe perspective.

Itincorporatesenhancing theproceduresbywhichexisting pharmaceuticalsare made.It concentrates on medication revelation and improvement and is concerned with the disengagementoftherapeutic operatorsfoundinplants.Itlikewiseaddstotheformationofnew manufactured medication mixes. In a groupwith researchersfrom diversefields, including scholars, toxicologists, pharmacologists, hypothetical physicists, microbiologists, and biopharmacists, moderndiagnostic strategies areutilized toorchestrate and test new medication items furthermore to build up the most financiallysupportive and environment sustainable method forproduction.(Lemke et al,2013)

ThustherealmofMedicinalChemistry haswitnessedasteady increasewiththeemergenceof medical disciplines, and ensured progress in mass spectrometry and population pharmacokinetics.A colossalneedtopropagatethe findingsinthe mostunsurpassed manner through ahandy medium for researchers, academicians, laboratory personnel, chemists, druggists, andpathologists alikehas been feltinthe recentyears.

The partof therestorative scientific expertinmedicationrevelationhasexperiencedrealchanges inthepreviously25years,primarily inlightofthepresentationofadvancements,forexample, combinatorialscienceandstructure-basedmedicationoutline.As therapeuticscientificexperts withover 50years ofconsolidated experiencetraversing the previousfourdecades,weexamine this changing part utilizing samples from our own particular and others' experience. This

chronicledpointof viewcouldgivebitsof knowledgeintohowtoenhancethepresentmodelfor medicationdisclosureby helping thetherapeuticscientistrecapturetheimaginative partthat added to past victories.

Flowresearchinmedicationdisclosure fromtherapeutic plantsincludesa multifaceted methodology joiningorganic,phytochemical,natural,andatomicstrategies.Therapeuticplant medication revelation keeps on giving new and imperative leads against different pharmacologicaltargetsincluding malignancy,HIV/AIDS,Alzheimer's,junglefever,and torment.A fewcommonitemmedicationsofplantbirthplacehaveeither as oflatebeen acquaintedwiththeUnited Statesbusinesssector,including arteether,galantamine,nitisinone, and tiotropium, or areasofnow included in late-stage clinical trials. (Cada,2001)

1.2 Abrief accounton medicinalplants:

1.2.1 Natural product in healthcarepractice:

"Naturalproduct"meansacompoundwhichdoesn'thaveanyprimarybiochemicalroleorthe role isunknown inthe organismfromwhichitgetsproduced.The molecular weightsof these moleculesarevery small.Thesecanalsobecalled"secondary metabolites"becausetheyare synthesizedfromtheorganismintheformofbiologically activechiral. Thedrug likeeffectof naturalproductsareusually higherthanthesyntheticproductsbecausenaturalproductshavethe tendencyoftransportanddiffusionin thecellularlevelastheyare producedinorganisms.Inthis way, itcan modifythe cellular activityin thepathologic condition.

Naturalproductshave more protonatedamine,free hydroxylions,more single bondsandmore fusedringsthansyntheticcompounds.Naturalproductscanactasadrug intheformofnon- modification or they act also as a lead compound after some semisynthetic modifications. (Lemke etal, 20013)

1.2.2. History ofmedicinal plants:

Therearemanyancientsystemsofhealthcarewheremedicinalplantsarewidelyused.Inthe Indian subcontinent, Ayurveda andUnaniarethemostpopularsystems.

Ayurvedasaysthatit'sfirstrecordedtreatmentwasmentionedinCharakaSamhita(900B.C).It has 8 sections which are divided into 150 chapters and itdescribes about 341 medicinal plants.In 14thcentury SarangdharaSamhitawaswrittenby Sarangdharawhichisasystemized materiamedicaofAyurvedaandisalsoavery importantadditioninthissystemasthebookis consisistof 2500verses.In1200AD.a book calledChikitsaSarsamgrahawhichwaswritten by Vangasena. This book states themedicinal properties of iron, mercury, sulphur and copper.

Unanimedicinalsystemhavecontributedalottopharmacy.Thissystemismainly developedby theArabians.Rhaze,Albucasis,Avicenna,IbnZuhurarevery popularnameswhohadgreat contributionsinUnani.Arabsmade thepharmaceuticalproductsmoreuser friendly.Alcohol, Camphor,Myrrh,Coffeae.t.caresomemedicinalArabicwordsthatarecommoninEnglish. (Ali, 2006).

Theutilization ofrestorativeplantsinEuropein thethirteenthandfourteenthhundredsofyears wasinviewoftheteachingofmarksorcomparativecreatedby Paracelsus(1490-1541AD),a Swisschemistanddoctor.Asperthisconvention,allplantshadsomesign,givenby themaker, whichshowedthesickness,indicationorailingorganforwhichtheywereplanned.Atypical caseof this convention incorporates ginseng-panaxginseng(Murray,1994).

The SouthAmericannationshave furnishedtheworldwithnumeroushelpfulrestorativplants, developednormally intheirwoodsandplantedinthetherapeuticplantgreeneryenclosures.The African individuals have been relying upon plant-based solutions more than some other landmass' kin.TotheextentthesouthAsian informationconcern,themostpunctualnotice oftherestorativeutilizationofplantsintheIndiansubcontinentisfoundintheRigVeda(4500-1600BC),themostseasonedbookinthelibraryofhumankind.Thisbookgivesmuchdataon thetherapeuticutilizationofplantsin theIndiansubcontinent.There aremorethan8,000plant speciesinSouthAsiawithownrestorativeuses(Switzeretal,2003).Inthisway verifiably itis clear that South Asiais home to numerous rich Traditional frameworks ofmedication (TSM).

1.2.3. Significanceof natural product:

Nowadayschemical synthesis is donemore asmanycomputational andcombinatorial techniques havebeendevelopedalong withbiotechnologicalprocesses.Fromthisperception,wecansay that,naturalproductis notthatimportantasthepastbutwe shouldnotforgetthatsemisynthetic agentsaredesignedbasedonthestructureofnaturalproducts.Thesesecondary metabolitesthat are collectedfromdifferentorganismsare ahugesourceofmoleculeswithoutstanding diversity which helps to continuethe drugdiscoveryprocess.

Powerfulleadmixescanbegatheredfromextraordinary creatures.Amidpickingplantsand differentlivingbeingsforthetrialreason,principally fivemethodologiesareutilizedarbitrary screening,choice of taxonomic gatherings, chemotaxonomic methodology,data oversaw methodologyand determination byanethnomedicalmethodology. (Lemkeet al,2013)

Therearevery smallnumberofanimalproductsthataretraditionally popular.Othernatural products like antibiotic and hormones are also veryessential.

Theideaofcancerpreventionagentsisfastlymaking upforlosttimeandmostrecent examinationhasdemonstratedthata number ofhomegrownsubordinateshave fabulouscell reinforcementactivity.BacopamonniericontainsbacosidesAandB andbacosideAwillbeanin number cell reinforcement, which decreases a few ventures of free radical harm. Coleus forskohlii[forskolin], Grapeseed[proanthocyanidins],Camelliasinensis[polyphenols], Huperziaserrata [huperzine],Pinusmaritima [Pycnogenol],Boragoofficinalis [gamma linoleic acid],Vincaminor [Vinpocetine]arepotentialcellreinforcements.Theplantisabiosynthetic research center for concoction mixes, as well as ahugenumberof mixes likeglycosides, alkaloidsandsoon.Theseapply physiologicalandremedialimpact.Theintensifiesthatarein chargeofrestorativeproperty ofthemedicationaretypically optionalmetabolites.Anorderly investigationof anunrefinedmedicationgraspsthroughthoughtof essentialandauxiliary metabolitesinferredasanaftereffectofplantdigestion system.The plantmaterialissubjectedto phytochemicalscreeningfor thelocationofdifferentplantconstituents.Withonsetoflogical explorationinherbals,itisgettingtobeclearerthatthe therapeuticherbshavea potentialin today'smanufacturedtime,asquantitiesofprescriptionsaregetting tobesafe.Asindicatedby oneevaluationjust20%oftheplantvegetationhasbeenconsideredand60%ofengineered

prescriptionsowetheirbirthplacetoplants. Oldinformationcombinedwithinvestigative standardscangotothebleedingedgeandfurnishuswithintensesolutionsfordestroy the illnesses. (Evans, 2005)

1.2.4. Pharmacologically active compounds in medicinal plants:

Althoughsyntheticprocesshasvastlydeveloped,plantshavealwaysbeenthemostreliable source of therapeutic agentsfor differentdiseases.Variousactive constituentsobtainedfrom plantsare steroidalhormones,glceryrrhatic acid(anti-inflammatory);Morphine(sedative); quinine(anti-malarial);rescinamine, ajmalicine, vinacamine(vasodilator);vincristine, podophyllotoxin(anticancer); caffeine,theobromine,theophylline(CNS stimulant) and so on. (Ali, 2006).

1.2.5. Development of newdrug frommedicinal plants:

Thebeginningyearsof21stcentury hadalotofopportunity torenewtheeffortstowardsthe discovery ofnewsecondary metabolites.Compoundsarebeingcollectedfromanimalsand marinesource.Ontheotherhand,thedesigning processesofnewsyntheticcompoundsare developingvery fast.Newresearchprojectsarebeing launchedsothatleadcompoundscan be found forHIV/AIDS,tuberculosis,hepatitis-Candsometropicaldiseases.Thereisnoneedto assumethat,after200yearsofinvestigation,theprospectsoffinding ofnewdrugsof natural originarenearing exhaustion.Muchhopeforsuccessisremaining inthistypeofendeavor. (Lemke etal,2013)

1.2.6. Approaches to researchand drug discovery:

Medicinalchemistry hasagreatinfluencingrole inmoderndrugdiscovery.Itfinishedthetimes ofstraightforwardblend;andpresentedthoseofcomplexengineeredroutinesandinnovations, for example,combinatorialscience(combichem),microwavehelpednaturalunion(MAOS)and high-throughput(HTS)organicscreeningstrategiesthathaveadvancedtheeveryday lifeofa physicist.Thesenewtechnologiesarehelpinghimtoattainhisgoalmuchmorerapidlyinthe

discovery process.Itdictateshowdrugsmustbedesigned,synthesizedandpurifiedsuccessfully inordertoaideinthefirststepofdevelopment. AMedicinalchemistcombinescomprehensive knowledgeofthe syntheticchemistry, medicinal chemistry, and biologyliteraturewith the abilityto drivethe project forward.(Evans, 2005)

1.3 Rationaleof thework:

The objectiveofthisworkisto prepare methanolicextractofthepeelsof*Mangiferaindica*and analyzeitscytotoxicand hypoglycemiceffect ofmango peelwhere theextractisinducedinthe animal model.

1.4 Presentstudyprotocol:

Inthisanalysis,wearegoingtodry andgrindthepeels.Thenitwillgothroughmethanol extraction. Byusingtheextract of thepeel cytotoxic and hypoglycemictests willtakeplace.

Table-1.1:Work designofthewholestudy.

Extraction	**PartSolvent** Peel Methanol
Phytochemical Screening	Different quantative tests to find outthe presenceof chemical constituents.
Pharmacological Study	**Method**
Activity Cytotoxic Hypoglycemic	BrineShrimp Cytotoxicity Bioassay(In-vitro) Glucose TolerenceTest (In-vivo)

1.5. TheAnacardiaceaefamily:

Treesorshrubsofthisfamilyarewitholeo-resinouswhichisoftenacridjuice.Leavesare simpleorcompound.Flowersaresmallorregular,1sexualor2sexual. Calyxis3-5partiteor sometimes accrescent. Stamens are equalto thenumberofpetals.Fruitsare usually1-5celled,1-5seeded,stonesometimesdehiscent.Seedsareexalbuminous,embryoisstraightorcurved. There are60 Generaand500 species belongto this family. (Blatteret al, 2008)

1.6. Description of the plant:

The plantof mangiferaindica isa large spreadingevergreentree witha heightof 15m.Allparts areglabrousexcepttheinflorescence.Leavesarecrowdedattheendsofthebrancheswhichare 12.5-25by 3.8-7.5c.m.Theleavesareoblongoroblonglanceolate,shining,basenarrowed. Flowersare5m.mlongwithadisagreeableodorthatarearrangedinamany floweredpubescent panicleslongerthantheleaves.Ovaryisglabrous.Drupesare large,fleshy.Stoneiscompressed, fibrous and veryhard..(Blatteret al, 2008)

MangosfitinwiththesortMangiferaofthefamily Anacardiaceae.ThesortMangiferacontainsa fewanimalgroupsthatbearconsumable organicproduct.Alarge portionoftheorganicproduct treesthatarenormally knownasmangosfitinwiththespeciesMangiferaindica.Theother consumableMangiferaspeciesbyandlargehavelowerqualityleafyfoodsnormallyalludedto aswildmangos.Mangohasgottentobenaturalizedandadjustedallthroughthe tropicsand subtropics.Agreat partof thespreadandnaturalizationhashappenedinconjunctionwiththe spreadofhumanpopulaces,andasbeingwhatisindicated,themangoplaysanimperativepart intheeating routineandfoodofnumerousassortedsocieties.There aremorethan1000named mangomixedbagsallthroughthe world,whichisa demonstrationof their worthtomankind. Mangoisatypicalpationursery treeallthroughthetropics.Atthepointwhenready,this scrumptiouspastry naturalproductisespeciallyhighinvitaminA.Theorganicproductis likewise eatengreen, handled into pickles,pulps, sticks,and chutneys,andissolidified or dried. Theorganicproductislikewiseacriticalwellspring ofsustenanceforfeatheredcreatures,bats, creepycrawlies, and wellevolved creatures. (Bally,2006)

1.7. Distributionofplant:

TheclassMangiferabeginsintropicalAsia,withthemostnoteworthynumberofspeciesfound inBorneo,Java,Sumatra,furthermore,theMalay Peninsula.Themost-developedMangifera species, M. indica (mango), has its birthplaces inIndia and Myanmar.

Mangoispresently developedallthroughthetropicalandsubtropicalworldforbusinessorganic productgeneration,asagreeneryenclosuretree,andasashade treeforstock.InthePacific area, allmangoswerepresentedfromdifferentpartsoftheworld.The mostpunctualrecorded presentationsintoHawai'iwereearlierto1825;inany case,mostacquaintanceswiththePacific islandshavehappenedinthecourseof recentyears.CoupleofotherMangifera speciesare found inthe Pacific. Mangiferagedebe,M.minor, furthermore,M.mucronulataare found inthe SolomonIslandsandM.minor inMicronesia, however these either don'tnaturalproductor the organic product is unpalatable. (Bally, 2006)

1.8. Taxonomic hierarchyof mangiferaindica:

Kingdom: Plant

Division:Magnoliophyta.

Class: Magnoliopsida.

Subclass: Rosidae.

Order: Sapindales.

Family: Anacardiaceae

Genus: Mangifera.

Species: indica.

➢Botanicname:*Mangiferaindica*linn.

➢Common names:

Idele (Palau),kangit(Chuuk,Pohnpei), mago(Niue, Samoa, Tuvalu),manako(Hawaii), manggo, am(Fiji),mangko(Kiribati),mango (English),mango(Tonga),mangot,mangue,manguier (French),mangueira(Yap),aam,am,amb(Hindi),ampleam(Tamil),bobbiemanja, kanjannamanja,maggo,manggaboom,manja(Dutch), mamamuang(Indochina),mamung (Thailand), manga,mango(Spanish),manga,(Portuguese), manga,mempelam,ampelam (Malaysia),mangga (Tagalog),mangga,mempelam (Indonesia), mango(Ilokano)mango(New Guinea, Pidgin), Mangobaum(German), mwàngx(Laos), paho(Bisaya)(Philippines), svaay (Cambodia), tharyetthi(Myanmar),xoài (Vietnam). (Bally, 2006)

2.Reviewofliterature:

2.1 *Mangiferaindica*:

2.1.1 Traditional useof *Mangiferaindica*:

Asweknow,thetraditionalmedicinalplantsare themainweaponstotreatseveraldiseasesused inancientsystemsof treatmentlike AyurvedaandUnani.Differentpartsof theplantsof *Mangiferaindica*isalso used to curedifferent diseases in thesesystems.

The barkofthetree isusedin Ayurveda totreat diarrhea anditalsohelpstostopvomiting.Itis considered to be useful againsthemorrhagefrom lungs, intestine and uterus.

Thefruitisanastringentandalsofoundhelpfulasastimulanttonicindebility ofthestomach. Theripefruitisconsideredasalaxativeandwidely usedby thepeoplesufferingfromhabitual constipation. Both greenand ripe fruits areused as anti-scorbutic after sundrying.

InUnani,the seedsareused asastringenttothebowels,inchronic diarrhea andalsoasagood collyrium.

Leavesare usedtotreatpilesinAyurveda.The smokeofthe leavesisappliedtostophiccough, roughness of the throat.

Resinousjuiceofthebarkisusedtotreatsyphilis,diarrhea,dysentery,scabiese.t.cinthe Malabarcoastalarea.Root,bark, stemandleaf–thecombinationisusedtocure snake bite.In westAfrica,pilesistreated byyoung barkof*Mangiferaindica*.In Madagascar,thebarkis consideredasanastringent.Fruitisusedasa tonicfor mucousmembrane inAmerica.Fluid extractistotreatdiphtheria inthisregion.InBrazil,the flowers of*Mangiferaindica*isdried and powderedinsize of tea leaves.Thenitisused forthecatarrhof bladder.The fume of the powder is applied againstmosquitoes. (Blatteret al, 2008).

2.1.2 Phytochemical constituents of*Mangiferaindica*:

2.1.2.1 Mangiferin:

Mangiferinisaxanthoneandxanthonesareprobably themostintensecancerpreventionagents known.They are thoughttobemoreintensethanbothvitaminC orvitaminEandare nowand theninformally alludedtoassuper cancerpreventionagents.Xanthonesarewarmthstable particles.Mangiferinisby andlargecalledC-glucosylxanthonewhichisgenerally conveyedin higherplants(Sanchezandothers2000) whereitgivesassurancetomaker plantsagainst distinctivetypesofstaticandelementanxietiesincluding devastationofpathogenic microorganisms(Muruganandanandothers2002).Itisapharmacologically dynamic phytochemicalanda characteristic polyphenoliccancer preventionagentpresentinthe bark, organic products,roots,andleavesof*Mangiferaindica*.A couple ofother therapeuticplants prescribedintheIndianarrangementofprescriptionfor treatment of variousimmunodeficiency maladies (Scartezziniand Speroni 2000).

Mangiferin(C2-β–D–glucopyranosyl-1,3,6,7-tetrahydroxyxanthone)(Figure 1)wasinitially confinedfrom*Mangiferaindica*leavesand fromthebark homomangiferin(1,6,7-trihydroxy-3-methoxy-2-C-β-D-glucopyranosyl-xanthone) wassegregated. Aquantitative estimationonthe dried leafandbark materialuncoveredthatthemangiferinsubstancewashigher inthe mango bark than inthe leaf andanaccountgivenof the co-eventof the 3xanthones(mangiferin, isomangiferin,andhomomangiferin).They separated2xanthonesintheleavesalongsideathird xanthone, iso-mangiferin(1-,3-,6-,7-tetrahydroxy-4-C-β-D-glucopyranosyl-xanthone),which hadbeeninitially recognizedinAnemarrhenaas-phodeloides.Mangiferinsubstanceofmango mashwasobservedtobearound4.4mg/kg,seedbit42mg/kg(Ahmedandothers2007).In driedmangopeelitwas1690mg/kg(Table1).Inthemangostembark, mangiferinwasthemost bounteousphenoliccompound,assessedataround71.4g/kg .Fromaworldwideperspective,xanthonesare justknownnotlimitedconveyance.Thenagain,mangiferinhasamoreextensive circulation (recorded insideof 12 families),and inside ofthe Anacardiceaefamily.

Numerous analystshaveset up mangiferinas theconceivabledynamic compound of mango (Mangiferaindica.)stem bark and leaf remove andcredited thegreater partofthe organic exercises ofthe concentrates to it (Sanchezand others 2000).Fromthe different studies doneon

mangiferinand theconcentrates from mango leaves, bark, and blossoms, itdisplayanextensive varietyof pharmacological impacts:cellreinforcement, anticancer, antimicrobial, against atherosclerotic, antiallergenic, mitigating, pain relieving,and insusceptiblemodulator among numerous others. Mangiferin has been explored in-vitro forits cellreinforcement (Rouillardand oth-ers 1998), immuno-animating, and antiviral properties (ZhengandLu 1990)

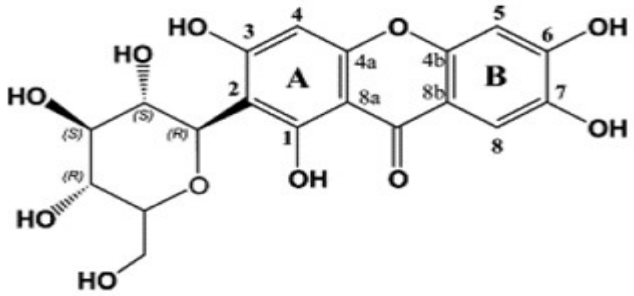

Fig-2.1.:Structureofmangifrin

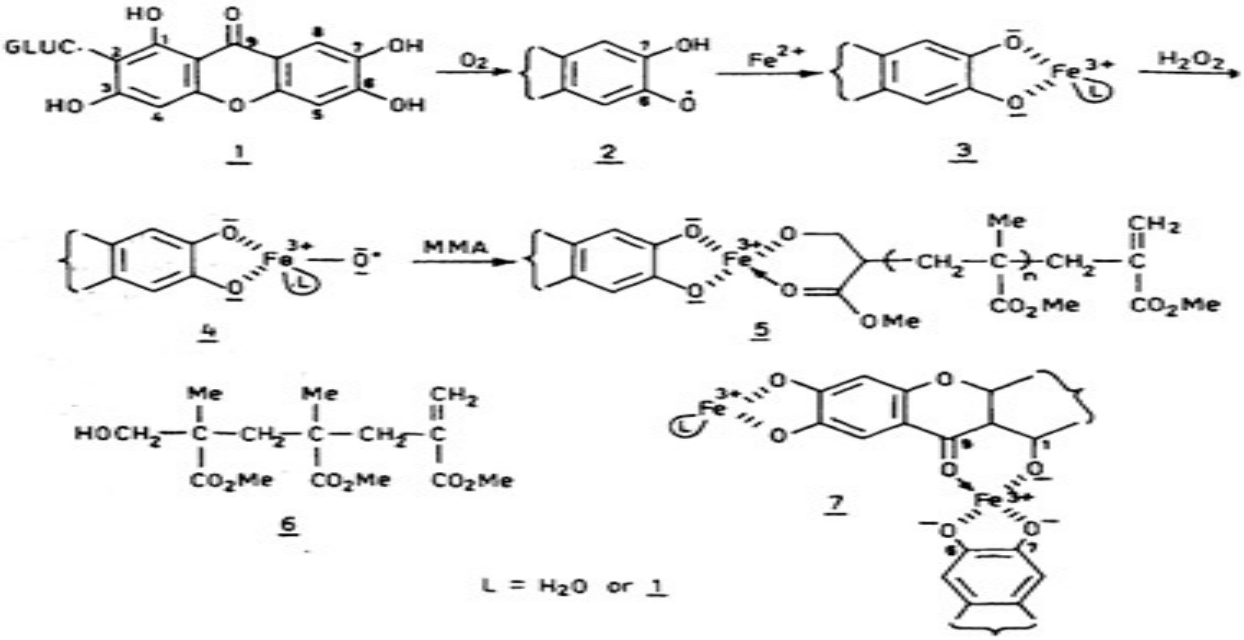

Fig-2.2: Mechanismofaction ofMangiferin.

Table 2.1: Phenolic compounds inmango peel (mg/kg)on drymatterbasis.

SerialNo	Compounds	Amount (mg/kg)
1.	Mangiferin	1690.4
2.	Mangiferingallate	321.9
3.	Isomangiferin	134.5
4.	Isomangiferingallate	82.0
5.	Quercetin 3-O-galactoside	651.2
6.	Quercetin 3-O-glucoside	557.7
7.	Quercetin 3-O-xyloside	207.3
8.	Quercetin 3-O-arabinopyranoside	101.5
9.	Quercetin 3-O-arabinofuranoside	103.6
10.	Quercetin 3-O-rhamnoside	20.1
11.	Kaempferol 3-O-glucoside	36.1
12.	Rhamnetin3-O galactoside/glucoside	94.4
13.	Quercetin	65.3
14.	Total phenolics	4066.0

Source: Berardiniand others (2005a)

2.1.2.2. Flavonoids:

Fromourdiet,wegetplentifulamountofflavonoids.Once upona time,itwasconsideredas vitamin-C2.Onthebasisofthedegree ofoxidation,flavanoidscanbeclassifiedintoseveral classes.They areflavones,isoflavones,flavanones,flavinols,anthocyaninsandproanthocyanins.

Amongalltheflavonoidsthatare presentinourdiet,the mostimportantone isquercetin.Itis available innumerousproductsof the soil.Itisfound innourishmentsasO-glycosideswhere sugarisboundatC3position(Hertoget.al1992).Theflavonoidsthathavediphenylpropane skeleton (C6-C3-C6) are considered to have high oxidative properties alongside hostile to

mutagenic,againstcancer-causing;mitigating andagainstunfavorably susceptibleimpacts (Hollmanet.al1996).Theflavonoidsthoseare availablein*Mangiferaindica*–catechin, epicatechin, quercetin, isoquercetin, fisetinandastragalin (Harborne1994)

a. Catechins:

Catechinisonekind offlavonoidthatisdividedintoseveralclasseslikeepicatechin, epgallocatechin,epicatechingallateandgallocatechin**.**Catechinsarereally helpfulforthe bettermentofhumanhealthastheyhavetheability toscavengefreeradicalandantioxidant activity(Augustyniaket.al 2005).

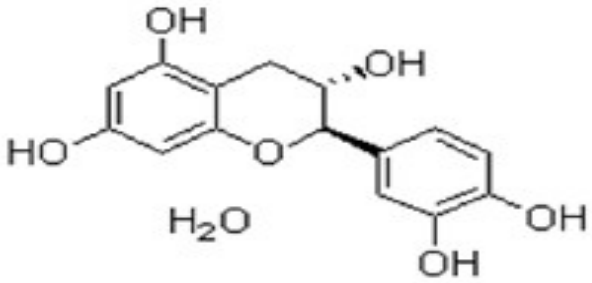

Fig-2.3: ChemicalstructureofCatechin.

It hasadditionallytheabilitytoanticipate congestiveheart disappointment,growth (yamanakaet.al 1997), myoglobinuricintense renal disappointment. Catechin canrespond with H2O2 straightforwardlyor keepthe Fenton response in the middleof Fe2+ and H2O2 to shape hydroxylradicals.Itcanlessen the measureof H2O2 that is incited byT-cellreceptorenactment. Alongtheselines, itcancontrol the responsiveoxygen species pathwayagainstactuation of affected cellpassing.(Hernandezet.al 2007)

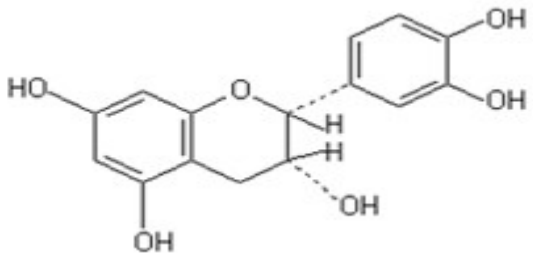

Fig-2.4:Chemical structureofEpicatechin.

b. Quercetin:

Quercetinsare theflavonoidsthatare thereasonbehindthecolorsofmostofthefruitsand vegetables.Quercetinisusually presentinthefruitsasglycosides.Thequality ofquercetinin *Mangiferaindica*isvery high which isshowninto table-1(Bardinietal2005b). Quercetincan decreasethe risk of breastcancer,coloncancer, leukemia,etc.Ithasbeen foundthat, high measurementsofquercetincanrepressthecellexpansionincolongrowth.Inany case,atlow dosages, the cellexpansion rategets expanded (Wenigeret al 1986).

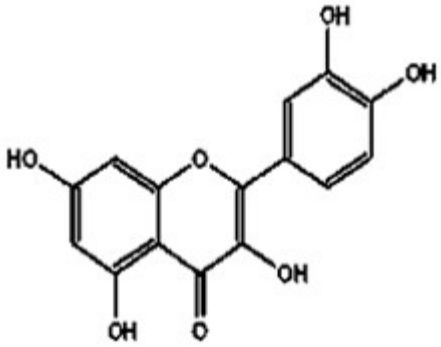

Fig-2.5:Chemical structureofQuercetin.

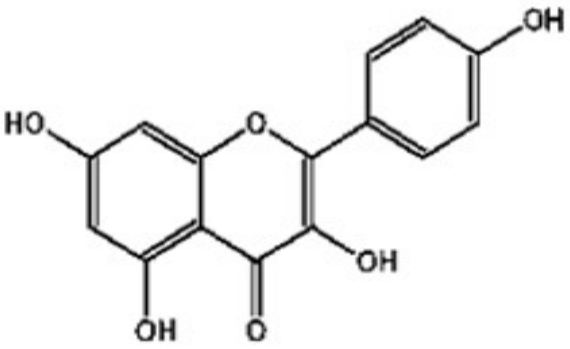

Fig-2.6:Chemical structureofKaempferol.

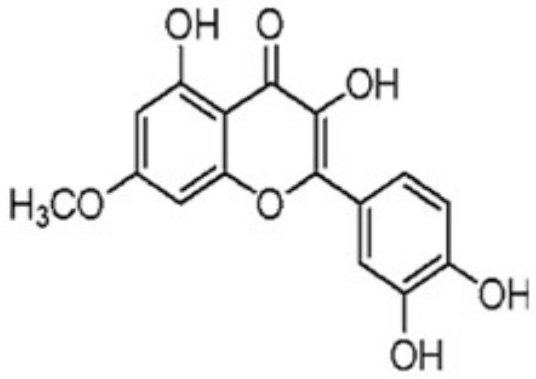

Fig-2.7:Chemical structureofRhamnetin.

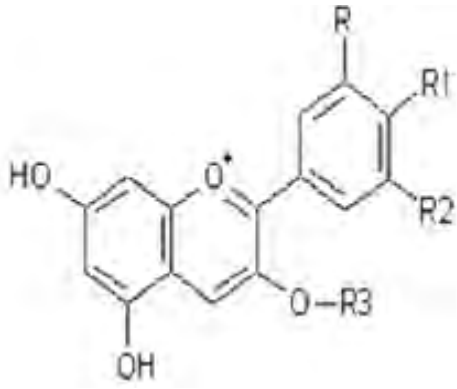

Fig-2.8:Chemical structureofAnthocyanin.

2.1.2.3 Phenolicgroup:

Phenolicacidsareampleinplantfoods.Theidentifiedphenolicacidsare Gallic acid,benzoic acid,gallicacidpropyl ester, benzoic acidpropylester, 3,4-dihydroxybenzoic acid,Gallic acid methylestere.t.c(Rastraelliet.al2002).Usually they areconvertedintoglucoseafter esterification. Gallicacidisfoundfromgallotanninsandother phenolicacidscan beobtained from the oxidation of galloylresidue from ellagitannins(Schemdaand Williamson 2000). Tanninsthatcangounderhydrolysisare usually thederivativesofphenolic acids.Theiractivity isnotasmuchascondensedtannins.Hydrolyzabletanninsare methylgallate,digillicacid,gallicacid, ellagicacid, alpha gallotannins, beta-gallotanninse.t.c. These compoundsare found in

differentmangoplantpartslikepulp,seeds,leaf,peel,steamand bark.Gallotanninsare toxic.Its amountthatispresentinfruitsisreallynegligible.Gallotanninsandellagicacidsarepermitted touseasfoodadditives. Invivohydrolysabletanninscanreducethebiologicalvalueofprotein richfoodsby formingcomplexwithit.7.5%tanninsthatarepresentinmangoseedsandkernel containhydrolysabletannins.Beforeintroducingthe tanninsintohuman body,the toxic effects areneeded to be minimized byusingwaterblanchingmethod (NigamandMitra1982).

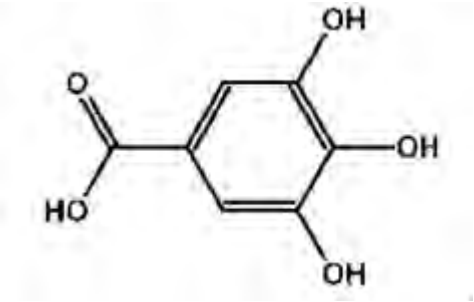

Fig-2.9:Chemical structureofGallic acid.

Ellagicacidisthedimericformofgallic acidandbothofthemexistas freeandboundfrom. Gallicacidcontainshydroxylgroup andcarboxylic acidgroup whichhelpittoconvertitselfinto digallicacid.Gallicaciddoesnothaveany astringenteffectbecauseitdoesnotcombinewith proteinmolecules.Theamountofhydrolysabletanninsismuchhigheringreenmangoesthan therippedones.Tannicacidistheformofgallicacidanhydrate.Butitis morepowerful astringentthangallicacid.Itcanalsocoagulatealbumin,gelatin.Beforeabsorption,tannicacid is converted intogallicacid into theGIT (Maxwell,1997).

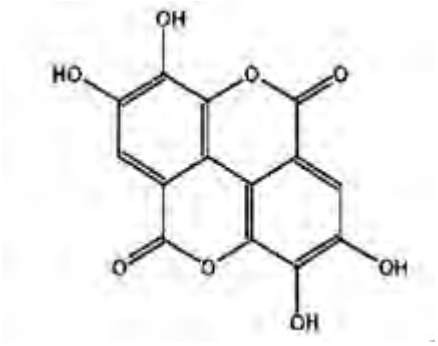

Fig-2.10:Chemical structureofEllagic acid.

In diversein-vivo and in-vitro studies, ithas been demonstrated thatgalliccorrosivehasa few helpful impacts like cell reinforcement, calming, hostile to microbial, against mutagenic,against growth and so on. Ellagiccorrosivecontains melded 4-ringpolyphenol and itis available in high sumin mango organicproduct.It is waterdissolvable and that is the reason itis less demanding to be consumed bythe animals.13-cis-retionic corrosive cango about asanadversaryagainstthe preventiveimpacts ofellagic corrosive (Dinneen,2006).Less measureofellagitanninsaremore successful in human bodythan bigger measureofimmaculateellagiccorrosive. Methylgallateand propylgallateareother known subordinates ofgalliccorrosive. Theyhave exceptionally solid cancer prevention agent properties.In studies, ithas been shown thatthesetwo subordinatescan possiblyconflict with herpes simplexinfection. Theycan likewiseforestall attachment ofhuman leukocytes,grip ofdiseasecells with vascular endothelial cells furthermore development of intestinal microscopic organisms.In-vivo studies havedemonstrated that, methyl amidepropylgallate subsidiaries can bring about theharm ofdeoxyribosesugar in vicinityof H2O2 and ferric EDTA(Alam et al, 1990).

Benzoicacidhasavery simplestructure.Itcontainscarboxylgroupboundtoabenzenering. Benzoicacidanditsderivativescanplay avitalroleinthemitochondrialpermeability transition (MTP).Inhumanbodies,benzoicacidhelpsto stimulatetheproductionof amino acid.When benzoic acidisatitsmaximumconcentrationin-vivo,itcancause highrateof gallic production. Hippuricacidandbenzylglucuronicacidareurinary metabolitesofbenzoicacid(Pernetand Meyer, 1997).

Photocatechuricacid(3,4dihydrobenzoicacid)isa formofdihydrobenzoicacidwhichhasbeen found inthe stembarkof*Mangiferaindica*.Dihydrobenzoicacidhastherapeuticeffectslike antipyretic,analgesic, antrheumaticetc.Besidesthat,thederivativesofthisacidhave anti-mutagenic,anti-carcinogenic,anti-fungal,anti-bacterial,anti-oxidantproperties(Wegneretal 1996).

Apart fromallthe statedphenolicacids,*Mangiferaindica*alsocontainscaffeicacid,ferulicacid, cinnamicacid.Allofthemhavestronganti-oxidanteffect(Ahmedet.al2007).Allof these phenolicacidsarevery importantinpharmaceuticalandnutritionscienceduetotheirsignificant contributions.

2.1.3 Bioactivity of*Mangiferaindica*:

2.1.3.1 Antioxidant activity:

Indiverse in-vivoandin-vitrostudies,it hasbeendemonstratedthatgalliccorrosive hasa few helpfulimpactslike cellreinforcement, calming,hostile tomicrobial,againstmutagenic, against growthandsoon.Ellagiccorrosivecontainsmelded 4-ring polyphenolanditisavailableinhigh suminmangoorganicproduct.Itiswaterdissolvableandthatisthereasonitislessdemanding tobeconsumedby theanimals.13-cis-retioniccorrosivecangoaboutasanadversary againstthe preventiveimpactsofellagiccorrosive(Dennis,1988).Lessmeasureofellagitanninsare more successfulinhumanbody thanbiggermeasureofimmaculateellagiccorrosive.Methylgallateandpropylgallateareotherknownsubordinatesofgalliccorrosive.Theyhaveexceptionally solid cancerprevention agent properties. In studies, it has been shown that these two subordinatescanpossibly conflictwithherpes simplexinfection.Theycanlikewiseforestall attachmentofhumanleukocytes,gripofdisease cellswithvascular endothelialcellsfurthermore developmentof intestinalmicroscopicorganisms.In-vivostudieshave demonstratedthat,methyl amidepropylgallatesubsidiariescanbringabouttheharmofdeoxyribosesugarinvicinity of H2O2 and ferric EDTA(Aruomaet.al 1993).

Two essential conditions must be satisfied foracancer prevention agent:

(i) Thecompoundoughttobeavailableinlowfocuseswithrespecttothesubstratetobe oxidized.

(ii) Thespeciescomingaboutbecauseofitsoxidationmustbesteadythroughintra-atomic hydrogen holding adjustment (Halliwell1990).

ThedefensivecancerpreventionagentcapacitiesofaMangiferaindicastembarkconcentrate was examined in-vivo in OF1 mice(Sanchezet al.2000).

2.1.3.2. Immunomodulatoryeffect

Thegreaterpartofthequalitiesovercommunicatedinaggravation,forexample,thoseencoding expert incendiary cytokines, chemokines, grip atoms and provocative compounds, contain

κBlocales insideof theirpromoter recommending that thesequalitiesare controlled dominatingly by theatomicelementkappaB(NF-κB)(Christmanetal.2000;Aggarwaletal.2006).The initiation ofNF-κBand its related kinasesasIκBαkinase (IKK) depends much of thetime on the creation ofROS (Mannaet al. 1998;Kumar and Aggarwal).

2.1.3.3. Anti-inflammatory activity

Provocative proceduresinclude awide rangeofsubstance middlepeople,forexample,nitric oxide(NO)andprostanoidsincorporated by inducibleisoformsofNOsynthase(NOS)and cyclooxygenase(COX-2),separately.Vascularoccasionsconnectedwithanincendiary response incorporatedilatationof the littlearterioles,bringingaboutexpandedbloodstreamand porousness.Mangiferaindicademonstratesthismovement.(Brionesetal.2002;GarciaandStein 2006; Zeilhofer 2007).

2.1.3.4. Anti-allergic activity

SortIunfavorably susceptiblereactionisfundamentallyintercededbypole cellsenactedthrough theconnectionof theirsurface receptors (FcεRI) withparticular mixes,for example,anIgE-boundantigen.Theseconnectionsbeginaprogressionofbiochemicaloccasionsbringing about thearrivalofnaturally dynamicgobetweensthatcauseunfavorablysusceptibleresponse(Chang andShiung2006).Basophils,eosinophils,lymphocytesandneutrophilsareadditionallyincluded intheunfavorably susceptiblereaction.Increaturemodelsofsensitivity,Mangiferaindicaaltogether (i) lessensIgElevelsinovoalbumin-vaccinatedmice; (ii) restrainsinactive anaphylacticresponses;(iii)diminishes histamine-impelledcutaneous response;(iv) diminishes theexacerbate48/80-incitedhistamine dischargefromrodentpolecells; and(v) repressesthe lymphocyte proliferativereaction to ovoalbumin incitement (Rivera et al.2006).

2.1.3.5Anti-diabeticactivity

Diabetes mellitusspeakstoa progressionofmetabolic conditionsconnected withhyperglycemia andbroughtaboutby imperfectionsininsulindischargeaswellasinsulinactivity.Insort1 diabetes,pancreatic β-cellsare demolishedviaauto-safeincendiarycomponents.Sort-2diabetes isacomplexmetabolicissueconnectedwithβ-cellsbrokennessandwithdifferingdegreesof

insulinresistance(Dinneen2006).Asoflate,ithasbeenaccountedforthatlong standing hyperglycemiawithdiabetes mellitusprompts thearrangement ofcutting edgeglycosylatedfinal itemswhichareincludedintheeraofROS,promptingoxidativeharm,especially toheartand kidney(Rolo and Pal-meira2006).

2.1.3.6. Antiviral activity

Zhuetal.concentratedonin-vitrothatthe impactof*Mangiferaindica*againstHerpessimplex infectionsort2.Itdoesn'tstraightforwardly inactivateHSV-2yethindersthelateoccasionin HSV-2replication(Zhuetal.1993).In-vitromangiferinwaslikewiseready tohinderHSV-1 infection replication insideof cells (Zhenget al. 1990).

2.1.3.7. Antibacterialandantifungal activities

Inanin-vitro agar dispersionprocedure,mangiferindemonstratedmovementagainst7bacterial species.They are-*Bacillus*pumilus,B.*cereus*,*Staphylococcusaureus*,S.*citreus*,*Escherichiacoli*, *Salmonellaagona*, *Klebsiellapneumonia*.

Itlikewise acted against1yeast(Saccharomyces*cerevisiae*) and4growths (*Thermoascusaurantiacus*,*Trichodermareesei*,*Aspergillusflavus*andA.*fumigatus*)(Stoilovaet al. 2005).

2.1.3.8 Monoamineoxidase-inhibitionactivity

Themonoamineoxidase-hinderingactionwasexploredongrown-uppaleskinnedpersonrats and mice. *Mangiferaindica*peelremove (100 mg/kg)-

(i)Significantlypotentiates hexobarbitone (100 mg/kg) narcosisbyabout 80% (dozingtime);

(ii) Plienates the apoptosis and turns aroundthe sedation incited byreserpine;

(iii) Potentiates amphetamine (25 mg/kg) poisonous qualityin total rats. (Rivera et.al, 2006)

3.MaterialsandMethods:

3.1. Preparationofplant extracts:

3.1.1. Collection andidentification

At to begin with, with the assistanceof an extensivewriting auditof*Mangiferaindica*from Anacardiaceaefamilywas chosen fortheexamination. The entireplant was gatheredfrom Dhaka, Bangladesh and distinguished by the taxonomist of the national herbarium of Bangladesh,mirpur, Dhaka. The voucher examples of the plants have been store in the herbarium for further references.

3.1.2. Plantmaterial preparation

After collectionthe fruitof*Mangiferaindica*,thepeelwasseparatedandsundriedfor abouta week. After dryingthe peel to optimumamount, itwas converted into powder byusing agrinder. About 1 kgpowder was collected for extraction procedure.

3.1.3. Extraction procedure:

1. 318gmangopeelwassoakedin700mlmethanolinonejarand332gmangopeelwas soaked in 700 ml methanol in another jar.
2. Thejarwas closed and kept forsevendays.
3. Themixturewas stirredwelleveryday.
4. Duetosomesolventlossbyevaporation,about200mlmethanolwasaddedineachjar and the jarsweresealedwith foil paper.
5. Aftersevendays,thesoakedpeelwasfilteredbycloth,cottonand“whatman”filter paper.
6. Thefilterwasdriedinrotaryevaporatorat50^0Cfor40minuteswheretherotationspeed was 100 rpm.
7. Thenitwaspouredinabeakerandkeptinthefumehoodforfurtherevaporationofthe solvent.
8. Afteraweek,stickyextractwasobtainedwhichwaskeptinadryplaceinnormal temperature.
9. The crude extract was used forphotochemical andpharmacological evaporation.

3.2. Phytochemicalscreening:

Table-3.1 :Different tests performedto check different compounds.

Serial No	Nameof the test	Procedure	Observation	Conclusion
1.	Lead acetatetest	3mlextract was added tofew drops oflead acetate.	Yellow colored precipitatewas formed.	Flavonoids were present.
2.	Foam test	0.5gm extract was takenin a 10ml vialand 2 mlwaterwas added. Afterthat, itwas shakenproperly.	Foam was formed that lasted formore than 10 minutes.	Saponins were present.
3.	Tannin test	1mlferric chloridewas taken in a test tube and 2mlextract was added there.	Blue-black colored precipitatewas formed.	Tannins were present.
4.	Steroid test	1mlextract was treatedwith 5 drops of concentrated sulfuric acid.	A redcolored indication was given.	Steroids were present.
5.	Terpenoid test	2mlchloroform, 3ml concentrated sulfuricacid and 5 ml extract was taken in a test tube.	Browncolored indication was given.	Terpenoidswere present.
6.	Glycoside test	5mlextract, 2ml glacial aceticacid, 1 dropof ferric chlorideand 1 ml of concentrated sulfuricacid was taken in a test tube.	Browncolored ringwas formed.	Glycosides were present.
7.	Wagner's test	1mlextract was treatedwith few drops of Wagner'sreagent.	Red colored precipitatewas formed.	Alkaloids were present.
8.	Molish's test	1 mlextract was treatedwith few drops of Molish'sreagent.	No violet ring was formed.	Carbohydrates wereabsent.

3.3. Pharmacologicalinvestigation (In-vivo) :

3.3.1 EvaluationofBrineShrimpLethalityBioassay :

Principle:

Forrapidandcomprehensivebioassay ofthecompounds,BrineShrimpLethalityBioassayisa populartest(McLaughlin,1998;Persoone,1980).By takingafterthisstrategy,bioactivity of unadulteratedmixescanbetried.Inthisstrategy,Invivolethality inBrineshrimpnaupliiis utilized as a great screenforscreeningof thebioactiveitem.

Bioactivemixesareregularlypoisonoustolivingbody whenhigherdosagesareadministered. Brineshrimplethalitybioassay helpstoevaluatethetoxicologicalaspectofthebioactive compounds. This assay is an important tool for first round assessment of cytotoxicity of bioactive compounds. This methodis aimed at the determination of LC_{50} Values of experimentingcompounds.

Asitisafastprocess(24hour),cheapandobligesnouniquehardware orasepticsystem,Brine ShrimpLethalityBioassaystrategy isconsideredasagreatdealmorebetterthanother cytotoxicitytestingtechniques.Itcanbeperformedwithanexpansivenumberoflivingbeings forfactualapprovalandamoderately littlemeasureoftest(2-20mgorless).Otherthanthat,it doesn't oblige creature serum. This technique shows pharmacological exercises like antimicrobial,antiviral,pesticidalandagainsttumorandso forthofthemixesalongside cytotoxicity(Meyer, 1982; McLaughlin, 1988).

Inthisstudy,thecytotoxicity measurewasperformedonsalinesolutionshrimpnaupliiasper Mayertechnique(Hossainetal.,2004). Saline solutionshrimpnaupliicanbecreatedbybring forththeshrimpeggsinmimickedoceanwater.Togetcovetedamassingsof thetestexamples, Dimethylsulfoxide(DMSO)wasincludedlegitimatesum.Thenaupliiwerecountedby visual reviewand were takeninthosepre-checkedtesttubescontaining5mlofreenactedoceanwater alongsidethespecimen.Atthatpointthetesttubeswereleftfor24hourstookafterby the numbering ofthesurvivorsfollowing 24hours(Meyeretal,1982).Theseinformationwere handled in astraightforward system forprobitinvestigation togaugeLC50values.

Materials

- *Artemiasalina*leach (brineshrimp eggs)
- Seasalt -sodium chloride, NaCl
- Small tank with perforated dividingdam to hatch theshrimps
- Lamps to attract shrimps
- 20 Test tubes.
- Test tubeholder
- Pipettes, micropipette (50-200 µl)
- Glass vials (10ml)
- Magnifying glass
- Dropper.
- Test samples containing experimentingcompounds

Preparationofseawater

38gmseasalt(NaCl)wasweighedanddissolvedinoneliterofdistilledwater.Thenthis solution was filtered fortwo times anda clear solution ofsimulated seawater was obtained.

Hatching ofbrineshrimps

*Artemiasalina*leach(brine shrimpeggs) wascollected frompetshopswhichwasused asthe test organism.Simulatedseawaterwaspouredinasmalltankandshrimpeggswereaddedtoone sideofthetank.Lightwasprovidedby alamponthetank.Twodayswereallowedtohatchthe shrimp.Thematuredonesareknownasnauplii.Constantoxygensupply wascarriedout throughoutthehatchingtimeby afixedmotorinthetank.Withthehelpofadropper,10living shrimps wereadded to each of thetest tubes containing5 mlof simulated seawater.

Preparationoftest samples

- 10cleantesttubesweretaken.Thesewereutilizedfortenuniquefixations(onetesttube forevery focus) oftestspecimen.Theothertentesttubesweretakenforcontroltest (dissolvable dimethyl sulfoxide).

- 4mgofeachtestsamplewastakenanddissolvedin100µlofpuredimethylsulfoxide (DMSO) ina glassvial anditisconsideredasstocksolution.Then50 µlof solutionswas taken in no-1 test tubeeach containing5 ml ofsimulated seawater. Thus thefinal concentrationofthepreparedsolutioninthefirsttesttubewas400µg/ml.Thenaseries ofsolutionshavingvaryingconcentrationswerepreparedfromthestocksolutionby serialdilutionmethod. Ineachcase50µlofsamplewasaddedtotesttube.The concentrations of the obtained solutions in each test tube are stated in Table-2. Ten nauplii was added to each test tube.

Table-3.2: Different concentrations ofdifferent testtubes contacting thesample.

Test tubeno	Concentration
1	400
2	200
3	100
4	50
5	25
6	12.5
7	6.25
8	3.125
9	1.56
10	0.781

Preparationofcontrol group

Controlgatheringsareutilizedasapartof cytotoxicitystudytoapprove theteststrategies.These gatheringshelptoassessandcontrastthe outcomesanda standard cytotoxicspecialistand guaranteethattheoutcomesacquiredarejustbecauseofthemovementofthetestspecimensand

theimpactsoftheotherconceivableelementsare invalidated.Inthisstudy,justNegativecontrol gatheringwas utilized.

Preparationofthe positive control group

Positivecontrolinacytotoxicity study isagenerally acknowledgedcytotoxicoperator.The reasonofusing positivecontrolgatheringistothinkabouttheconsequenceofthetestoperators withtheoutcome gotfromthepositive control.Inthepresentstudy,Vincristine Sulfatewas utilizedasthe positive control.Measuredmeasure of the Vincristine Sulfate wasbrokendown in DMSOtogetabeginningcentralizationof20µg/mluponwhichserialweakeningwere made utilizingDMSOtoget10µg/ml,5µg/ml,2.5µg/ml,1.25µg/ml,0.625µg/ml,0.3125µg/ml, 0.15625 µg/ml,0.078125 µg/ml,0.039 µg/ml.Atthatpointthese diverse focusarrangementsof thepositivecontrol wereaddedtothepre-checkedtesttubescontaining tenliving brackishwater shrimp naupliiin 5 ml mimicked ocean water to get thepositive control.

.

Preparationofthenegative control group

50µlofDMSO wasaddedtoeachofthreepre-checkedtesttubescontaining5mlofmimicked oceanwater and10saline solutionshrimpnauplii.Thesetesttubeswere utilizedascontrol gatherings.Ontheoff chancethatthesaltwatershrimpsinthesetesttubesdemonstrateaquick deathrate, then the testisconsideredasinvalidbecuase the naupliipassedonbecause of some reasonotherthancytotoxicity ofthemixes.Itmightbepassedonbecauseofthedangerous activityof thedissolvableor thearranged recreated seawater.

Counting ofnauplii

Following24hours,thetesttubesweretreviewedprecisely utilizinganamplifyingglassandthe quantity ofsurvivorsofshrimpnaupliiwerenumberd.Therate(%)ofmortality wasfiguredfor everyweakeningof focus.

3.3.2 EvaluationofHypoglycemic Activity

Principle:

Diabetesmellitusisanendocrinedisordercommoninbothmaleandfemale.Itisoneofthe major public healthissue inBangladesh.Once upona timeitwasbelievedthatitwasa disease thathasarrivedfromthewest.Butwiththemodernizing andurbanizing ofthepopulationinour country,ithasbecomeanendemic disease.Ayurveda demonstratesthatseveral herbalmedicines preparedasoralformulationhavebeenprovenpotentagainsthypoglycemicactivity.(Zeilhofer, 2007)

Astandoutamongstthemostadequateroutinesforassessing thehypoglycemicactionisglucose resilience test(GTT).Itisa therapeutic testwhereglucose isgivenandbloodtestsandafter that gatheredashort time later to decidehow quicklyit is cleared from theblood. Thetestis typically usedtotestfor diabetes,insulinresistance,receptivehypoglycemiaandrarerissueofstarch digestion system.Thistesthas been performed throughouttheyearsfordifferentpurposes with distinctive standard dosagesofglucose,diversecoursesof organization,diverse interimsand lengths of time ofexamining, and different substances measured notwithstandingbloodglucose.

Inthisstudy,hypoglycemicimpactofmethanolicconcentrateofthepeelofMangiferaindicaat 200mg/kgand400mg/kgmeasurementswereanalyzed&contrastedwithrelativewiththatof control andstandardgathering.

Experimental Animals

Swiss-pale skinnedperson miceof either sex, measured 25gm in normal were gotten from the creatureplaceof State UniversityofBangladesh situated in Dhanmondi, Dhaka, Bangladesh. Theywerehoused in standard polypropyleneconfines andkept under controlled room temperature (24 ± 2ºC; relative dampness 60-70%). All outsixmicewereutilized to perform this examination.

Fig-3.1: SwissAlbino mice (Source: http://goo.gl/KtoCkf)

Experimental Design

Six test creatures were haphazardly chosen and isolated into two gatherings signified as gatheringI,aggregateIIcomprising of3miceinevery gathering.Every gatheringgotdistinctive measurementsofconcentrate.Precedinganytreatment,everymousewasmeasuredappropriately andthedosagesofthetestexamplesandcontrolmaterialswere balancedasneedsbe.Asitwas hardtowatchthebiologicreactionofthreemiceatonceaccepting sametreatment,itwas importanttodistinguishsingularcreatureofagatheringamidthetreatment.Alongtheselines, thecreatureswerecheckedas1=Mice1,2=Mice2,3=Mice3,4=mice-4,5=mice-5and6= mice-6.

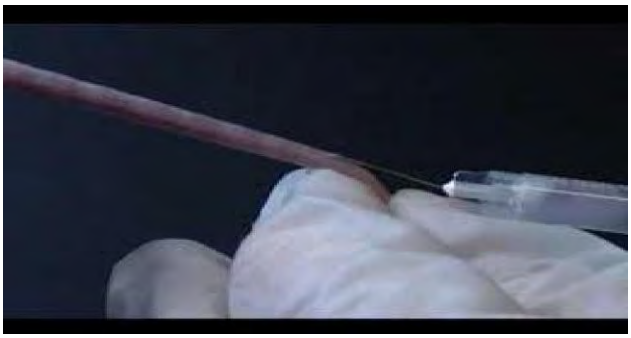

Fig-3.2:Pricking mice'stail. (Source: https://goo.gl/VDK4Zt)

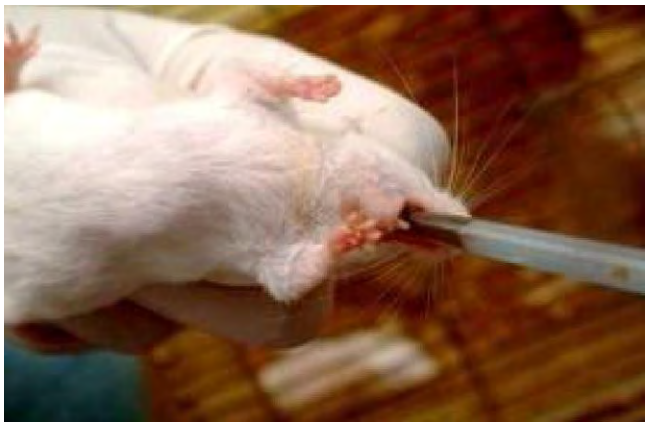

Fig-3.3:Oral administration(Source: http://goo.gl/PZG0gX)

Preparationofextract containingdosage:

- For200mgdose,24mgextractwastaken ina5mlvialwith0.8mldistilledwaterwasadded there.
- For400mgdose, 48mgextract was taken in a 5mlvial with 0.8ml distilled water.
- 2drops of Tween-80 was added in each vial to ensureproper mixtureof the extract.
- Then the extracts weredissolved in thevial byusingvortexmachine.

Preparationofglucose solution:

10gglucosewasdissolvedin100 mlwaterwhich wasusedtoincreasethebloodglucoselevelof the mice.

Procedure:

1. At zero hourtest samples, control (1%Tween-80 solution in saline) and Glibenclamide (in standardgroups)were administered orallybymeans of alongneedle with aball shaped end. Thetest samples were also administered orallyto thetestgroups.
2. After60 minutes,the tailof each mousewas pricked and blood was taken into the strip of diabetes measuringmachine. Current blood glucoselevel of everymousewas recorded.
3. 1mlglucosesolution wastaken in a syringeand administered orally.
4. After20 minutes,glucoselevel waschecked again and thedata was recorded.

5. Then 0.2mlextract wasgiven orally.(200mgdoseto mouse1-3 and 400mg doseto mouse4-6).
6. Bloodglucoselevel waschecked after 30,90 and150 minutes. Thedata was recorded in a chart.

4.ResultandDiscussion

4.1 Dataanalysisofhypoglycemic activity

The effects of methanolicextract of peel of *Mangiferaindica*at 200 and 400 mg/kgdoseto lower blood glucoselevel were observed asfollows to evaluatetheirhypoglycemic activity-

Table-4.1: Testmaterials usedinthe evaluationof hypoglycemicactivity ofcrude extract ofpeelof*Mangiferaindica*

Code no.	**Test Samples**	**Group**	**Identification**	**Dose(mg/kg)**
CTL	1% Tween-80&DMSO in normal saline	I	Control Group	0.1 ml/10 gof body wt
STD	Glibenclamide	II	Standard Group	10
ME 1	Methanolic extract of peel of *Mangiferaindica*	IIIA	Test Sample	200
ME 2	Methanolic extract of peel of *Mangiferaindica*	IIIB	Test Sample	400

Table-4.2: Plasma level ofglucose (mmol/L) ofmiceat different time

Code no.	0 minute		30 minute		90 minute		150minute	
	Data	Mean	Data	Mean	Data	Mean	Data	Mean
CTL	5.8 5.8 5.5	5.70	10.1 10.9 11	10.67	7.6 7.2 7.2	7.33	5.7 5.8 5.3	5.60
STD	4.1 4.2 4.2	4.17	3.6 3.7 3.9	3.73	3.3 3.6 3.7	3.53	3.6 3.2 3.1	3.30
ME 1	4.0 5.1 2.5	3.87	6.6 9.0 12.7	9.43	3.5 2.8 3.3	3.20	3.7 3.1 2.2	3.00
ME 2	4.9 3.7 5.2	4.6	5.1 13.3 8.0	8.8	2.7 5.7 3.7	3.97	2.4 5.1 2.8	3.43

Table-4.3: %reductionof plasma glucose level by testmaterials

Code no.	%reduction		
	After30minutes	After90minutes	After150minutes
STD	10.55	15.35	20.86
ME 1	-143.67	66.06	6.25
ME 2	-91.30	54.88	13.60

FromtheabovetableitisclearthatME1andME2havegreaterpercentagereductionvalues than STD after 30,90and 150 minutes of administration.

Table-4.4 :Hypoglycemic activity of crude extract of peel of*Mangiferaindica*

Code no.	Plasma level ofglucose (Mean)			
	0 minute	30 minute	90 minute	150 minute
CTL	5.70	10.67	7.33	5.60
STD	4.17	3.73	3.53	3.30
ME 1	3.87	9.43	3.20	3.00
ME 2	4.6	8.8	3.97	3.43

Statistical data evaluation

Thestandardt-Testwascarriedoutforthetestsamplesincomparisonwiththepositivecontrol and the statistical significanceof thedata was calculated.

Table-4.5: Statistical evaluationofthedata

Code No	t-Test value	DegreeofFredrom	Pvalue	Level ofsignificance
STD	3.0412	6	0.0228	Statisticallysignificant
ME 1	1.2775	6	0.2486	Not Statisticallysignificant
ME 2	1.2609	6	0.2542	Not Statisticallysignificant

4.2Dataanalysisofbrineshrimp lethality bioassay

The effects of methanolic extract of peel of*Mangiferaindica*at brineshrimp wereobservedas follows to evaluatetheir cytotoxic activity-

Table-4.6 : Effects ofin-vitro cytotoxicity test by BrineShrimpLethality Bioassay.

SampleConcentration (µg/ml)	**Log Concentration**	**Numberof NaupliiGiven**	**MeanPercentageof Mortality**	**VincristineSulphate**				**Negative Control**
			MethanolicExtract of Mango Peel	Concentration (µg/ml)	Log Concentration	No. Of Nauplii Dead	Percentage of mortality	Number ofNaupliisurvived
400	2.60206	10	90	40	1.60206	10	100	10
200	2.30103	10	60	20	1.30103	9	90	9
100	2	10	30	10	1	9	90	10
50	1.69897	10	40	5	0.69897	9	90	10
25	1.39794	10	20	2.5	0.39794	8	80	9
12.5	1.09691	10	20	1.25	0.09691	7	70	10
6.25	0.79588	10	0	0.625	-0.20412	4	40	10
3.125	0.49485	10	0	0.3125	-0.50515	0	0	10
1.56	0.193125	10	0	0.156	-0.80688	0	0	10
0.781	-0.10735	10	0	0.078	-1.10791	0	0	10

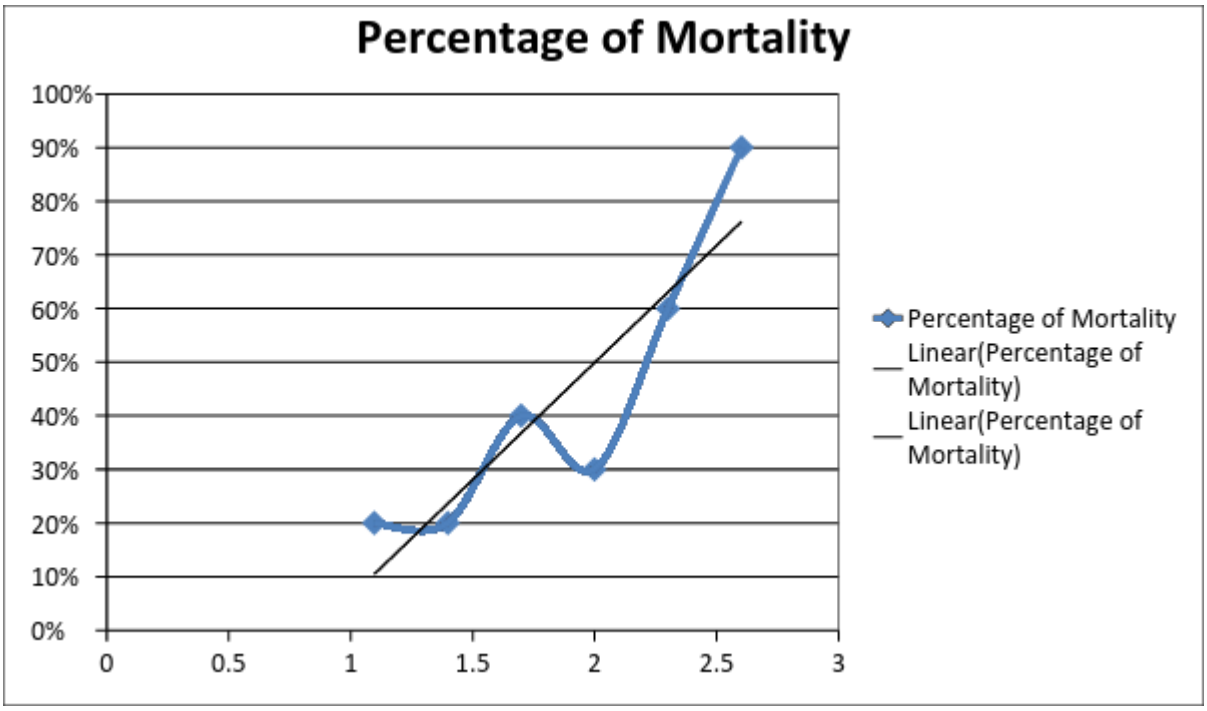

Fig-4.1: LinearCurveshowing thePercentageofMortality vs Log Concentration for MethanolicExtract ofMango Peel.

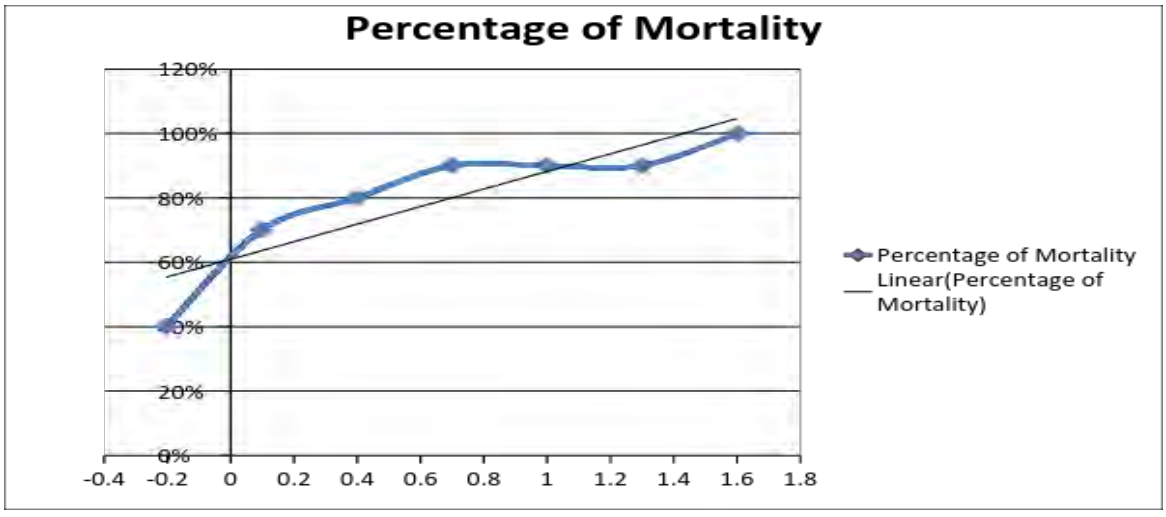

Fig-4.2: LinearCurveshowing thePercentageofMortality vs Log Concentration for MethanolicExtract ofMango Peel

Table-4.7: LC_{50}valuesofthestandardandmango peel extract

Sample code	Regression Equation	**LC_{50} (µg/ml)**	**R^2**
Vincristinesulphate	y=0.2729x+0.6093	0.41	0.7872
Mango peel extract	y=0.4366x-0.3741	2.04	0.8097

4.3 Discussion:

Vincristine SulfatewasutilizedaspositivecontrolandtheLC50esteemfor Vincristine Sulfate wasfound as0.41 µg/ml.Contrastedandthe negative control,Vincristine Sulfate (positive control)gavenoteworthymortalityandtheLC50estimationsofthedistinctivetestexamples were contrastedand negative control.

Asindicatedby thepreviously statedinformation,themangopeelextractdemonstratedhuge cytotoxicexerciseswiththeLC50estimationof2.04µg/mlindividually contrastedwiththe standard vincristine sulfate havingtheLC50estimation of 0.41 µg/ml.

Inanycase,fluctuatingdegreesoflethality wasseenwithpresentationtodiversemeasurement levelsoftheincorporatedmixes.Thelevelof lethality wasstraightforwardly relativetothe convergenceofthecompoundrunning fromcriticalwiththemostminimalfixationtovery noteworthywith themostnoteworthyfocus (400µg/ml).

Boththetwodosages200mg/kg and400mg/kg ofmethanolicextractshowedreductioninmean bloodglucoselevel.Althoughthereductionofmeanbloodglucoselevelof ME1andME2 werenotstatistically significantwhichcouldbebecauseoftheincreasedplasmaglucoselevelin 30minutes,reductioninmeanbloodglucose levelin90and150minuteswasfound.After administration ofglucose, itwas quite obvious that blood glucoseincrement would takeplace.

5.Conclusion:

Mangiferaindica,aplantofAnacardiaceaefamily knownasMangowasextractedwithmethanol showedmoderatehypoglycemicactivityandprominentcytotoxicactivity.Basedontheresults of hypoglycemicactivity,furtherstudies canbecarriedouttostrengthentheglucoselowering activity ofpeelsofmangousingStreptozotocinandAloxaninduceddiabeticratmodelsfor chronic studies.

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