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COMPARATIVE PROTEIN KINASE INHIBITORY ACTIVITY OF THREE ACACIA SPECIES GROWING IN NIGERIA

Augustine Ajibogun Ahmadu[1*], Bilqis Abiola Lawal[2], Aniefiok Sunday Udobre[3], Florence Tarfa[4], Adesegun Jubril Kashimawo[5], Patricia Odumosu[6]

[1]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Veritas University, Bwari-Abuja*
[2]*Department of Pharmacognosy and Drug Development Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo Nigeria*
[4]*Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and development, Idu Abuja, Nigeria*
[5]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce island, Amasomma, Yenagoa, Nigeria*
[6]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos Nigeria*

Significant improvements have been made in the field of drug discovery targets against cancer over the years, which have necessitated the search for novel chemical entities for the therapeutic treatment and management of cancer from natural sources.

In this present work, we report herein the kinase inhibitory effect of the crude chloroform extract and the partitioned soluble fractions of the hydroalcoholic stem bark extract of Acacia ataxacantha against a panel of disease related protein kinases and the comparison of the activity with that of Acacia nilotica and Acacia auriculiformis previously reported.

The extract and the fractions of the stem bark of Acacia ataxacantha were subjected to Kinase enzymatic activities performed in 384-well plates using the ADP-Glo assay kit as recommended by the manufacturer (Promega, Madsion,WI).

The result showed that chloroform extract was inactive, while the ethyl acetate fraction gave the highest activity against haspin kinase with IC_{50} of 0.1 µg/ml, followed by aurora B kinase with IC_{50} of 0.45 µg/ml, while against CDK2, CDK5 and CDK9 kinases, the IC_{50} were 0.6, 0.88 and 0.9 µg/ml respectively. The n-butanol fraction gave an IC_{50} of 0.59 µg/ml and 0.7µg/ml against haspin and aurora B Kinases respectively. Comparison with Acacia nilotica and Acacia auriculiformis previously investigated showed that the ethyl acetate fraction of acacia ataxacantha is the most active, while the chloroform extracts of the three acacia species were inactive. The results showed the potentials of the ethyl acetate soluble fraction of Acacia ataxacantha as probable source of inhibitors against Haspin, aurora B and CDKs kinases which might serve as lead for antimitotic agent against cancer.

Keyword: *Acacia species, Protein kinases, Ethyl acetate fraction, IC_{50}, Anticancer agent*

INTRODUCTION

It is known that 80% of the populace of the developing countries of the world depends on medicinal plants for their health needs,[1-2] natural products derived compounds play a significant role as the source of medicines due to the presence of secondary metabolites as purified extracts that exhibits multiple biological activities ranging from cytotoxic to cytoprotective activities which are considered inexpensive.[3] This has necessitated intensive

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*Corresponding author: Augustine Ajibogun Ahmadu, E-mail: ahmadu2001@yahoo.com

chromatographic studies as well as chemical characterization of active constituents which could unfold a novel chemical entities suitable for drug discovery.[4-6] Kinases are enzymes that catalyse the reversible transfer of activated phosphate groups from an energy–rich phosphate donor usually adenosine triphosphate (ATP), to substrates such as proteins, lipids, nucleosides and carbohydrates. Within this enzyme class, the protein kinases represent the largest sub family, accounting to about 2% of the human genome groups (d to as kinome.[7]The human genome is known to contain more than 518 protein kinases which constitute 1.7% human genes, as well as 20 lipid kinases.[8] Protein kinases catalyze the transfer of γ-phosphate from purine nucleotide triphosphate (ATP and GTP) to the hydroxyl group of their substrate by generating phosphate monoesters using protein alcohol groups (on serine and threonine residues) and or protein phenolic groups(on tyrosine residues) as phosphate acceptors. The 21st century has seen the emergence of protein kinase as primary targets for drug discovery against cancer as their inhibitors constitute about 33% of all efforts to develop pharmacologically active agents across the world.[9] With the array of therapeutic targets that could be selected for drug discovery, protein kinases are of special interest due to the significant role they play in intracellular signalling pathways which is involved in the pathogenesis of many chronic diseases like cardiovascular, inflammatory, autoimmune, nervous diseases as well as metabolic disorder, type 2 diabetes and cancer.[9-10]

Protein kinases can be classified as serine/threonine specific kinases which are made up of 385 kinases, tyrosine kinases which consists of 58 transmembrane receptor kinases and 32 non receptor kinases and a small group of dual specific protein kinases that phosphorylates both tyrosine and other kinases.[9] 2.5% of human protein-coding genes are kinases of which 50% of these genes are localized within cancer or other disease loci. Protein kinase inhibitors have been developed and tested for clinical application against various forms of cancer out of which 76 of these inhibitors have been developed.[11]The large scale genomic efforts couple with advancement in genome technology have led to an improvement in the clinical application of kinase inhibitors against cancer treatment.[12-13]53 small molecule kinase inhibitors were approved by the US FDA as of December, 2019, for use against malignancies such as chronic myeloid leukaemia, lung cancer and gastrointestinal cancer.[14]

Acacia belongs to the family Fabaceae and is a large genus consisting of over 1,400 species which are rich in chemical constituents such as flavonoids, gums and tannins.[15] The genus Acacia is ubiquitous in tropical and sub-tropical countries such as Nigeria, Ghana and Mozambique. *Acacia nilotica* Delile, is found naturally in sub–Saharan Africa including Nigeria, Egypt, Senegal, and Countries such as India, Burma and Australia.[16] Ethnomedicinal claims of this plant in hausa ethnomedicine of northern Nigeria reported the use of the leaves and bark to treat diarrhoea and inflammation. Literature reports from the family Fabaceae which include the genus Acacia has revealed that over 106 phytochemicals possess activities related to cancer treatment among which compounds from *A. nilotica* were described for anticancer activity and prevention purposes. The ear leaf acacia also referred to as *Acacia auriculiformis* is an important medicinal plant and widely distributed member of the family Fabaceae.[17] The aboringines of Australia are known to use an infusion of the bark to treat inflammation.[18] Antimutation as well as chemoprotective properties of the stem bark extracts have been reported,[19] while the ethyl acetate as well as the acetone fractions of this plant have been reported to possess antioxidant and free radical scavenging properties.[20,21] The plant *Acacia ataxacantha* is wide spread in sub-Saharan Africa including Nigeria, Benin and Kenya where various parts of the plant has ethnomedicinal applications in relieving ailments such as dysentery, cough, joint pains and pneumonia.[22-23]The stem bark of *acacia ataxacantha* has been reported to exhibit antioxidant, antifungal and antibacterial activities,[24-25] while triterpenoids and chromone have also been reported in this plant.[26-27]We have previously reported the isolation and structure elucidation of two novel flavonoids, a known flavonoid, steroids, ferulic acid ester and terpenoids from *Acacia nilotica, Acacia auriculiformis* and *Acacia ataxacantha* respectively, and their kinase activities.[16-17; 28-31] In this present work, we report herein the inhibitory activity of the ethyl acetate and 1-butanol soluble fractions of the stem bark of *acacia ataxacantha* against a panel of disease

related kinases as well as the comparative kinase inhibitory activity of the stem bark extracts and fractions of *Acacia nilotica* and *Acacia auriculiformis* which we have previously reported.

MATERIALS AND METHODS

Plant materials and Extraction

The plant material consisting of the leaves and stem bark of *Acacia ataxacantha* were collected in Zaria during the month of July and authenticated at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, and their vouchered specimen were deposited in the herbarium as reported previously.[30]

The powdered stem bark of *Acacia ataxacantha* (300 g) was extracted with 2.5 L of chloroform exhaustively using cold process of extraction to give 2.6 g of chloroform extract of *Acacia ataxacantha*. The dried marc was then extracted with 2x2.5 L of hydro alcohol to exhaustion using the cold process of extraction to give the crude hydro alcohol extract (24.6 g), of which 20.0 g was suspended in 400ml of water and partitioned with 2.5 L of ethyl acetate, and 2 L of n-butanol to give 2.6 g and 3.8 g of ethyl acetate and n-butanol soluble parts respectively.

Evaluation of Kinase enzymatic activities

The inhibitory effect of the chloroform extract, ethyl acetate and n-butanol soluble fractions against a panel of disease related protein kinases were tested using the following serine/threonine kinases: Hs (Homo sapiens) Cyclin-dependent kinase (Hs CDK2/cyclin A, Hs CDK 5/p25, Hs CDK 9/Cyclin T, Ssc (Sus scrofa) Glycogen synthase -3-alpha-beta (GSK 3α/β), Hs PIM 1, Hs haspin, Leishmania major casein kinase (Lm CK1), Ld (Leishmania donovani) TLK, Hs RIPK3 and Hs Aurora B. The assays were performed in a 384-well plates using the ADP-Glo assay kit in accordance with the recommendation of the manufacturer (Promega, Madsion,WI). Kinases were measured in appropriate dilutions of dimethyl sulphoxide (DMSO). The inhibitory activity of the kinase enzymes was measured using specified buffer, with either protein or peptide as substrate in the presence of 15 μM (^{33}-P) ATP (3,000 Ci/mmol;10 mCi/ml) in a final volume of 30 μL as described by (Bach et al, 2005)[32]. Full length kinases were used as specified.

RESULTS AND DISCUSSION

Result

The kinase activity of the extract and soluble fractions of *Acacia nilotica and Acacia auriculiformis* against disease related protein kinases which indicate the activity of the kinase remaining after treating the mentioned kinase with 50 μg/ml of the extract and fractions compared with control (DMSO) revealed that both the chloroform extracts of *A.nilotica* and *A.auriculiformis* were inactive against all the tested kinases as previously reported,[16,17] in contrast however, both the ethyl acetate and n-butanol soluble fractions were active and the IC_{50} of both fractions were investigated as previously reported .[23,24] Following the observed results for both *A.nilotica and A.auriculiformis*, the primary activity of the chloroform extract, ethyl acetate and n-butanol soluble fractions of the stem bark of *acacia ataxacantha* were not investigated rather the two fractions were tested over a wide range of concentrations in order to established the IC_{50} which were estimated from a dose response curve as summarised in **table 1**. The result revealed that the ethyl acetate fraction is the most active against the kinases tested, while the chloroform extract was inactive.

Discussion

Significant improvements have been made in the field of drug discovery against cancer. Despite the novel chemical entities available in the market for treatment and management of cancer, adverse effects such as organ damage, bone marrow suppression, hepatic, renal and gastrointestinal toxicities among others, have limit their uses,[33-34]hence the continuous quest for newer and effective drug candidates. Secondary metabolites found in natural products are known to possess diverse biological effects. These natural products are present in numerous medicinal plants, microorganisms and they are non-toxic and considered inexpensive.[3]From the result of the kinase activity investigated, it was observed that the chloroform extracts of the three acacia species were in-active against the tested kinases as compounds previously isolated from the chloroform extracts were reported to be inactive against all the tested kinases, these

compounds include Acanilol 2, a novel compound isolated from *Acacia nilotica* which was reported as a weak DYRK 1A inhibitor with an IC_{50} of 19 µM,[28]and from the chloroform extracts of *Acacia auriculiformis* and *Acacia ataxacantha*, a ferulic acid ester, lupenol and ά-spinasterol isolated were all reported to be in- active against all the kinase enzymes tested;[17,30]in contrast however, we have reported the kinase activity of (+)-catechin as a CLK1 inhibitor with an IC_{50} of 7.23 µM from the ethyl acetate fraction of *Acacia nilotica*,[16] while a tetrahydroxy flavone purified from the ethyl acetate soluble fraction of *acacia auriculiformis* gave an IC_{50} of 7.16 µM and 7.96 µM against DYRK1A and CDK9 respectively.[30] Betulin, a triterpenoid and an inducer of apoptosis purified from the ethyl acetate soluble fraction of the stem bark of *Acacia auriculiformis* exhibited inhibitory activity against multiple protein kinases.[31]Comparative kinase activities of the three Acacia species as shown in **tables (1&2) and Fig(1&2)**, revealed the activity of ethyl acetate fraction of *Acacia ataxacantha* when tested against a panel of disease related protein kinases. This fraction was shown to inhibit most of the kinases in micromolar range particularly against haspin (IC_{50} of 0.1 µg/mL) which is the best activity, followed by Aurora B kinase (0.45 µg/mL) and against the cyclic dependent kinases (CDKs 2,5 and 9 with IC_{50} of 0.88, 0.66 and 0.9 µg/mL respectively. CDK kinases are often overactive in human cancer due to their various genetic and epigenetic events which regulates their pathways resulting in uncontrolled proliferation.[35] Flavonoids have been reported to inhibit CDK 9, for example, wogonin, a flavonoid has been reported to inhibit CDK2, CDK7 and CDK 9.[36] The ethyl acetate fraction of *Acacia ataxacantha* inhibited all the CDK kinase tested **(Table 1)**. This observed activity of the ethyl acetate fraction which contain medium polar compounds, is likely to be due to the presence of polyphenols such as flavonoids. These phytoconstituents are known to possess a wide spectrum of activity against protein kinases. Quercetin is known to act on multiple kinase targets which are mostly involved in cell proliferation in cancer cells and are known to mediate their action via inhibition of the P13-Akt/PKB pathway by binding to P13 K¥ with IC_{50} of 3.8 µM. [37] Quercetin is also reported to mediate its kinase inhibitory activity by binding to the ATP binding pocket and protein substrate binding site and is also known to inhibit JAK2/stat 23 pathway via inhibition of hepatocellular carcinoma progression by modulation of cell apoptosis, invasion and autophagy all related to JAK 3 signalling pathway,[38] and it is also reported to be a well-known ATP competitive CK2 antagonist with IC_{50} of 0.2-1.8 µM.[39-40] Other flavonoids such as apigenin, chysoeriol have been reported to be a potent inhibitors of serine/threonine kinases.[41]The kinase activities of these flavonoids have been attributed to be due to the hydroxylation pattern as hydroxylated flavonoids such as myricetin, quercetin, were the most potent inhibitors in contrast to flavonoids with only two hydroxylation groups with no effect on the kinase activities. Acanilol 2 isolated from the stem bark of *Acacia nilotica* was reported as a weak DYRKIA inhibitor, while the methylated derivative (Acanilol 1) was found to be inactive against the kinases tested.[28] Haspin kinase is a haploid germ cell specific nuclear protein kinase. It is a serine/threonine kinase that associates with chromosome and phosphorylates threonine 3 of histone 3 during mitosis and it's over expression results in defective mitosis, its inhibitors have been reported to exhibit potent antitumour activity.[42] The results of this work as shown in **tables (1&2) and Fig (1&2)** revealed that both the ethyl acetate and n-butanol soluble fractions of the three acacia species showed profound activity against the haspin kinase enzyme thereby suggesting that the three acacia species might contain haspin kinase inhibitors which can be exploited for lead discovery against cancer. Aurora kinases belong to the family of mammalian serine/threonine kinases which have been reported to be involved in cell division particularly during mitosis and meiosis, this suggests that their inhibitors could serve as a lead for the development of drugs against cancer. [43,44] Over expression of aurora B kinase has been implicated in a wide variety of cancer such as prostrate, leukaemia and breast cancer,[45] and is also reported to induce abnormal chromosomes leading to genetic **i**nstability,[46]and subsequent development of cancer. The dual activity of a haspin and aurora B inhibitors as observed from the result of kinase inhibitory activity of *Acacia ataxacantha* might be a strategy to the development of a small molecule inhibitor

targeted against abnormal cell division in cancer development. This investigation has further confirmed the kinase inhibitory activity of ethyl acetate soluble fractions of the three Acacia species with that of *Acacia ataxacantha* which showed the highest activity against haspin, aurora B and CDK kinases.

Table 1: Effect of soluble fractions on Kinase inhibitory activity of *Acacia ataxacantha* (IC_{50} in µg/mL) [a].

Fractions	HsCdk2	HsCdk5	HsCdk9	SScGSK3	HsPIM1	HsHaspin	RIPK3	LmCLK1	LdTLK	AuroraB
Chloroform	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Ethyl acetate	0.88.	0.66	0.99	0.73	5.1	0.1	6.3	0.60	>50	0.45
n-Butanol	50.0	40.0	5.10	0.75	6.1	0.59	>50	10.0	>50	0.7

Values are reported in µg/ml (n=3; independent experiments). ATP concentration used in the kinase assays was 15 µM.

Table 2: Comparison of the kinase activities of the three Acacia species [b].

Protein kinases	NEA	NBU	UEA	UBU	TEA	TBU
Hs Cdk2	>50	>50	7.5	45.0	0.88	50
Hs Cdk5	31.0	>50	6.0	52.0	0.66	40
Hs Cdk9	8.0	11.0	4.5	17.5	0.90	5.1
Hs GSK3	11.0	8.1	9.0	15.0	0.73	0.75
Hs PIM1	3.9	15.0	5.0	20.0	5.1	6.1
Hs Haspin	1.2	2.2	1.0	1.3	0.1	0.59
Hs RIPK3	27.0	>50	30.0	17.5	6.3	>50
Lm CLK1	12.0	8.5	30.0	25.0	0.6	10.0
Ld TLK	>50	>50	>50	>50	>50	>50
Aurora B	2.9	3.0	5.0	5.0	0.45	0.7

b (IC_{50} in µg/mL, n=3, independent experiments**).**

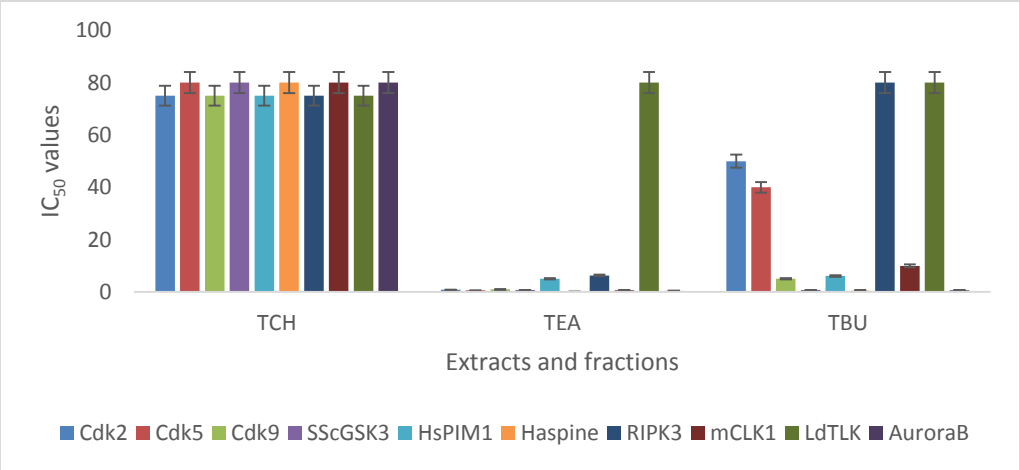

Fig. 1*:* IC_{50} of extract and fractions of *A. ataxacantha* against disease-related protein kinases.

Keys

TCH = *A. ataxacantha* chloroform extract.

TEA = *A. Ataxacantha*Ethyl acetate fraction.

TBU = *A. ataxacantha n*-Butanol fraction.

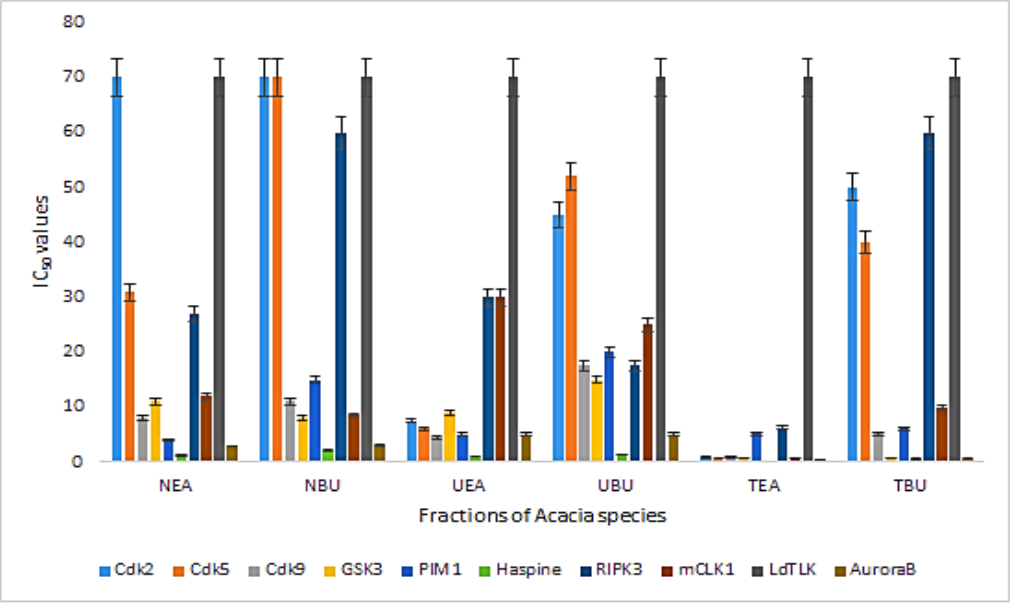

Fig. 2: Comparison of the kinase activities of the three Acacia species.

Keys

NEA = *A. Nilotica* ethylacetate fraction.
NBU = *A. Nilotica* butanol fraction.
UEA = *A. Auriculiformis* ethylacetate fraction.
UBU = A. Auriculiformis butanol fraction.
TEA = A. Ataxacantha ethylacetate fraction.
TBU = A. Ataxacantha butanol fraction.

Conclusion

This work has shown that the ethyl acetate soluble fractions of the three Acacia species contained active molecules that inhibited haspin, CDKs and Aurora B kinases in micromolar range and can be exploited as lead discovery for compounds targeted against cancer.

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مقارنة النشاط المثبط لبروتين كيناز لثلاثة أنواع من السنط تنمو في نيجيريا

أوغستين أجيبوغون أحمدو[1*] – بلقيس أبيولا لاوال[2] – أنيفيوك صنداي أودوبري[3] – فلورنس طرفا[4] – أديسيغون جبريل كاشيماو[5] – باتريشيا أودوموسو[6]

[1]قسم الكيمياء الصيدلانية والطبية، كلية العلوم الصيدلية، جامعة فيريتاس، بواري أبوجا

[2]قسم العقاقير وتطوير الدواء، كلية العلوم الصيدلية، جامعة إيلورين، نيجيريا

[3]قسم الكيمياء الصيدلانية والطبية، كلية الصيدلة، جامعة أويو، أويو نيجيريا

[4]قسم الكيمياء الطبية ومراقبة الجودة، المعهد الوطني للبحث والتطوير الصيدلاني، إيدو أبوجا، نيجيريا

[5]قسم الكيمياء الصيدلانية والطبية، كلية الصيدلة، جامعة دلتا النيجر، جزيرة ويلبرفورس، أماسوما، يناجوا، نيجيريا

[6]قسم الكيمياء الصيدلانية والطبية، كلية العلوم الصيدلية، جامعة جوس، جوس نيجيريا

انه قد تم إجراء تحسينات بارزة في مجال اكتشاف الأدوية ضد السرطان على مر السنين، مما استلزم البحث عن كيانات كيميائية جديدة لعلاج السرطان والتحكم به من المصادر الطبيعية.

في هذا العمل الحالي، نذكر هنا التأثير المثبط لبروتين الكيناز لمستخلص الكلوروفورم والأجزاء القابلة للذوبان المقسمة من المستخلص المائي الكحولي للحاء الساق لنبات *أكاسيا أتاكساكانثا* ضد العديد من بروتينات الكيناز المسببة للامراض ومقارنة هذا النشاط مع نشاط *أكاسيا نيلوتيكا* و *أكاسيا اوريكوليفورميس* المذكور سابقا.

تم إخضاع المستخلص وأجزاء لحاء ساق نبات *أكاسيا أتاكساكانثا* للأنشطة الأنزيمية لبروتين الكيناز التي تم إجراؤها في ٣٨٤ لوحة خلايا باستخدام مجموعة مقايسة ADP-Glo على النحو الموصى به من قبل الشركة المصنعة (Promega، Madsion، WI).

أظهرت النتائج أن مستخلص الكلوروفورم كان غير نشط، في حين أعطى جزء خلات الإيثيل أعلى نشاط ضد هاسبين كيناز بقيمة IC_{50} قدرها ٠.١ ميكروغرام/مل، يليه كيناز أورورا ب بقيمة IC_{50} قدرها ٠.٤٥ ميكروغرام/مل، بينما ضد كيناز CDK2، CDK5 وCDK9 حيث كانت قيمة IC_{50} هي٠.٦، ٠.٨٨ و٠.٩ ميكروغرام/مل على التوالي. وقد أعطى جزء n-بيوتانول IC_{50} قدرها ٠.٥٩ ميكروغرام/مل و٠.٧ ميكروغرام/مل ضد الهاسبين وكينازات أورورا ب على التوالي. أظهرت المقارنة مع القيم المذكورة سابقا لنباتي *أكاسيا نيلوتيكا* و *أكاسيا اوريكوليفورميس* أن جزء خلات الإيثيل من *اكاسيا أتاكساكانثا* هو الأكثر نشاطا، في حين أن مستخلصات الكلوروفورم من أنواع السنط الثلاثة كانت غير نشطة.وقد أظهرت النتائج إمكانات الجزء القابل للذوبان فى خلات الايثيل من *أكاسيا أتاكساكانثا* كمصدر محتمل لمثبطات ضد أنزيمات هاسبين و أورورا ب وCDKs والتي قد تكون بمثابة مثبطات التفتل المستخدمة لعلاج السرطان.